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POSTMORTEM EXAMINATION OF STILLBORN AND OF NEWLY BORN INFANTS

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The necropsy on the fetus or on the newly born infant is as different from the necropsy on the average adult as nepiology is from general medicine. To be successful, it requires of the pathologist special training and special interest in the causes of fetal and infantile deaths. The abnormalities are often so meager that they may be missed or, if observed, inaccurately interpreted. This paucity of spectacular abnormalities is largely responsible for the feeling that little is to be learned from necropsies on infants and that they may be relegated to the least experienced members of the staff. It is often more difficult to determine the cause of death in an infant than it is in an adult, and often it is impossible without access to a complete clinical history. No autopsy is adequate unless a complete microscopic examination is made of the organs and unless the head is thoroughly examined. The placenta, too, should be thoroughly investigated, for, particularly in regard to the stillborn infant, it may disclose the reason for the fetal death.

The newly born infant presents certain anatomic variations from the adult, and most pathologic conditions observed in the adult are nonexistent in the fetus. The most important causes of death in this period are those which prevent normal establishment of extra-uterine existence and which consequently never function again as a cause of death.

For recording on death certificates, all births are divided into two groups, stillbirths and live births. In the United States an infant is considered live born if it shows any signs of life after birth, such as beating of the heart or muscular movements, even if respiration is never established. For scientific investigation, a subdivision of each group is of aid in the classification of causes of death, stillbirths being divided into those occurring in the antepartum period and those in the intrapartum period, and live births into those in which breathing has begun and those in which respiration has never been established.

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Certain investigators, especially those in England, prefer to call stillborn the infant in whom the heart is beating at the time of birth but who never breathes, substituting the term dead born for the infant who shows no sign of life at birth. The reporting Committee of the League of Nations on Maternal Welfare and the Hygiene of Infants and Children of the Pre-school Age define a stillborn infant as any infant which has never breathed. The two groups are closely associated, and in general the same factors that cause stillbirth may produce death within the first few hours or days of life. Most deaths within the first few days are due to conditions extant before the infant was born. Any condition which kills the fetus in utero may in other instances affect the infant so that even if born alive it is unable to establish a satisfactory extra-uterine existence.

The most common causes of death in this period are interference with exchange of fetal and maternal metabolites between the two circulations, birth injuries, prematurity, congenital malformations and bacterial infections.

Interference with exchange of metabolites (most commonly a lack of sufficient oxygen in the blood going to the fetus) may be due to premature separation of the placenta, placenta praevia, insufficient size or massive infarction of the placenta, extreme anemia of the mother, toxemia with large amounts of nitrogenous material in the circulating blood, and compression or entanglements of the umbilical cord. Sudden interference with the circulation leads to extreme congestion of the viscera and numerous petechial hemorrhages on the surfaces of many organs, especially the thymus, lungs and heart. Occasionally larger hemorrhages occur within the substance of various organs. The presence of numerous petechiae in the absence of other pathologic conditions indicates asphyxia (inability of the fetus to obtain oxygen) as the probable cause of death. When circulation ceases more gradually, there are frequently no pathologic changes visible.

Injury may be sustained at birth owing to a variety of obstetric complications and occasionally is found after an apparently normal nonoperative delivery. Those conditions which lead to rapid or excessive change in the shape of the fetal head with resultant rupture of intracranial vessels are the most common causes. More rarely, abnormal manipulation of the neck may give rise to injury of the spinal cord, or undue compression of the fetal abdominal wall may lead to intraperitoneal hemorrhage from rupture of a viscus. The liver and adrenals are the organs most commonly involved.

Prematurity is not a recognized cause of stillbirth, for unless some complicating factor has produced the death of the infant the heart should be beating at the time of delivery. Premature onset of labor is not in itself enough to account for death. The fetus may be too

immature to be capable of carrying on an independent existence after it is once outside the uterine cavity and may be unable to produce even an initial expansion of the lungs, but it is, nevertheless, live born. In the very immature live-born infant (the so-called previable infant) death is due to anatomic inadequacy of development, which may be surmised on gross examination and definitely demonstrated microscopically. In infants who are moderately premature but with organs anatomically developed sufficient for function, there may be marked physiologic immaturity. It is in this group that nursing care is so important, and on its adequacy the fate of the infant usually rests. A premature infant is more adversely affected by any abnormal condition in the environment than one who is mature, and it is frequently difficult to decide the importance of prematurity in relation to other conditions as the basic cause of death.

Congenital malformations are easily demonstrable at autopsy, and ordinarily there is little difficulty in deciding whether or not they are the cause of death.

Bacterial infections, except syphilis, have a relatively unimportant role in the causation of either stillbirths or deaths during the first two weeks of life. Infection as a cause of malformation is largely discredited. The proved instances of transmission of infectious diseases from mother to fetus are comparatively few. Inspired infected amniotic fluid may presumably cause pneumonia. After birth the infant may succumb to any pathogenic bacteria which gain entrance to the body, but the isolated nature of its existence gives relatively little chance for exposure to such bacteria. If organisms do gain access to the body through any portal, the low resistance of the infant to bacteria frequently results in septicemia. Blood cultures made during life are of value but after death are less reliable. However, if an infection is suspected, cultures of the heart blood should be made. In interpreting cultural results the possibility of postmortem bacterial invasion should always be considered. Syphilis is an important cause of stillbirths, and the possibility that it is present should be investigated in every necropsy.

The effects of drugs and anesthetics administered to the mother during labor and delivery as factors in the production of fetal deaths are difficult to demonstrate definitely. Some drugs are transmitted through the placenta to the fetus, and when large amounts of narcotics are given late in labor the infant may be born with a definite depression of the respiratory center, which in itself may be sufficient to cause death. If this depression is coupled with prematurity, the effects of prolonged labor or other deleterious influences, it becomes even more important as a cause of inability to establish normal respiration. Under these circumstances no pathologic changes are ordinarily demonstrable in the body of the fetus.

Anesthetics which reduce the oxygen content of the blood of the mother may produce anoxemia in the fetus. This is particularly true when the anesthetic is administered over a prolonged period.

A factor which cannot be demonstrated by necropsy but which must be considered in any discussion of the causes of fetal death is one relating to the limitations of the germ plasm for continued growth. Both ova and spermatozoa vary in the degree of potential perfection of development. They may be so imperfect that they are incapable of maturation, fertilization or early development, or they may, on the other hand, carry the potentiality of perfect development and great longevity. When the cells are of intermediate quality, the degree of maturity which the fertilized ova are capable of attaining may be reached at any point of intrauterine or extra-uterine life. In some of these cases definite anatomic abnormalities will be present, but in another and probably larger group anatomic lesions will be absent.

Although it is certain that the union of an inferior ovum and spermatozoon may result in a fetus incapable of complete development, it is unwise for the pathologist, in attempting to give a specific cause of death in any given case, to wander too far into the realm of theory.

The number of cases in which lethal factors inherent in the germ cells are responsible for fetal death is almost impossible to estimate. Lack of complete knowledge of the effect of various endocrine, chemical and physiologic states of the mother in relation to the welfare of the fetus makes it possible that factors as yet nondemonstrable may have an important role in producing death of the fetus or of the young infant.

NECROPSY

Preliminary Examinations.—The preliminary examinations comprise determinations of sex, weight in grams and crown-heel and crown-rump length. A convenient instrument for measuring length consists of a flat board bearing a centimeter scale and having a fixed upright board at one end with a second attached movable upright board. The body is placed with the head against the fixed upright board, the movable one is placed firmly against the buttocks and feet successively, and the length is read directly on the centimeter scale. Owing to muscular relaxation, length is always greater after death than during life. Conversely, the dead infant invariably weighs slightly less than it did when alive.

Estimation of the degree of maturity of the fetus is frequently difficult. The menstrual history is notoriously unreliable as a criterion of fetal age. The wide variations in size of fetuses delivered approximately at term make it difficult to set up definite numerical standards. No two invesigators agree exactly on the normal length or weight. The centers of ossification of the various bones are probably more constant

than any other single finding, but Ballantyne [1] has expressed the belief that in relying on this evidence alone one may incur as much as a month's error in the calculation of the actual age. The time of appearance of the centers of ossification of various bones as given by a publication of the United States Children's Bureau [2] is found in table 1. Scammon and Calkins,[3] in common with most investigators, have stated that, of weight and length, length is the more constant in relation to the period of development. Cruickshank and Miller [4] found more agreement (78 per cent) between age as estimated by length and centers of ossification than between any other two criteria. Between weight and ossification centers there was 66 per cent agreement; between length and menstrual age there was 65 per cent agreement, while between weight and menstrual age there was only 57 per cent agreement. Weight and length in relation to age as given by different investigators may be found in table 2.

For the classification of infants in regard to degree of maturity at birth, at the Chicago Lying-in Hospital cognizance is taken, as a routine, of three factors: weight, length and menstrual age (table 3). The infant is placed in the group with the criteria of which at least two of its measurements correspond.

Because of the variation in size of infants at birth it is also difficult to determine normal weight for the organs. Organ weights depend in general on body weight, but there is considerable variation even in infants of the same weight and length. Average weights of organs of new-born infants at term may be found in table 4. The variation in size of the thymus at birth has long been a topic for discussion, but there is probably almost as much variation in the weights of other organs.

Maceration.—Maceration is probably the most common single abnormality found in stillborn infants. A fetus dying in utero is in a sterile fluid medium, so that the degeneration is of a different type from that which occurs when the infant dies after birth. It consists in softening and disintegration of tissue without putrefaction. The skin is involved first, and within a few hours after death the epidermis may loosen and be easily separated from the dermis. Occasionally the two layers are parted by accumulations of fluid (bullae), but more frequently they remain together until external manipulation separates them. The first

1. Ballantyne, J. W., quoted by Cruickshank and Miller.[4]

2. Growth and Development of the Child, White House Conference on Child Health and Protection, New York, Century Company, 1932, sect. 1, p. 67.

3. Scammon, R. E., and Calkins, L. A.: Growth in the Fetal Period, Minneapolis, University of Minnesota Press, 1929.

4. Cruickshank, J. N., and Miller, M. J.: Estimation of Fetal Age, the Weight and Length and the Weight of the Fetal Organs, Medical Research Council, Special Report Series, no. 86, London, His Majesty's Stationery Office, 1924.

TABLE 1.—*Time of Appearance of Centers of Ossification*[2]

	Week
Head	
Mandible	7th
Occipital bone (squamous portion)	8th
(lateral and basilar portion)	9th to 10th
Superior maxilla	8th
Temporal bone (petrous portion, mastoid and zygoma)	9th
Sphenoid (inner lamella of pterygoid process)	9th
(great wings)	10th
(lesser wings)	13th
(anterior body)	13th to 14th
Nasal bone	10th
Frontal bone	9th to 10th
Bony labyrinth	17th to 20th
Milk teeth (rudiments)	17th to 28th
Hyoid bone (greater cornua)	28th to 32d
Body	
Clavicle (diaphysis)	7th
Scapula	8th to 9th
Ribs	
5th, 6th, 7th	8th to 9th
2d, 3d, 4th, 8th, 10th, 11th, 9th	9th
1st	10th
12th (very irregular)	10th
Sternum	21st to 24th
Upper extremity	
Humerus (diaphysis)	8th
Radius (diaphysis)	8th
Ulna (diaphysis)	8th
Phalanges	
Terminal	9th
Basal 3d and 2d	9th
Basal 4th and 1st	10th
Basal 5th	11th to 12th
Middle 3d, 4th, 2d	12th
Middle 5th	13th to 16th
Metacarpals	
2d and 3d	9th
4th, 5th, 1st	10th to 12th
Vertebrae	
Arches	
All cervical and upper 1st or 2d dorsal	9th
All dorsal and 1st or 2d lumber	10th
Lower lumbar	11th
Upper sacral	12th
4th sacral	19th to 25th
Bodies	
From 2d dorsal to last lumbar	10th
From lower cervical to upper sacral	11th
From upper cervical to lower sacral	12th
5th sacral	13th to 28th
1st coccygeal	37th to 40th
Structural arrangement	13th to 16th
Odontoid process of axis	17th to 20th
Costal process	
6th and 7th cervical	21st to 33d
5th cervical	33d to 36th
4th, 3d and 2d cervical	37th to 40th
Transverse processes	
Cervical and dorsal	21st to 24th
Lumbar	25th to 28th
Pelvic girdles	
Ilium	9th
Ischium descending ramus	16th to 17th
Os pubis (horizontal ramus)	21st to 28th
Lower extremity	
Femur (diaphysis)	8th to 9th
(distal epiphysis)	35th to 40th
Tibia (diaphysis)	8th to 9th
(proximal epiphysis)	40th
Fibula	9th
Os calcis	21st to 29th
Astragalus	24th to 32d
Cuboid	40th
Metatarsal	
2d and 3d	9th
4th, 5th and 1st	10th to 12th
Phalanges	
Terminal 1st	9th
Terminal 2d, 3d, 4th	10th to 12th
Terminal 5th	13th to 14th
Basal 1st, 2d, 3d, 4th, 5th	13th to 14th
Middle 2d	20th to 25th
Middle 3d	21st to 26th
Middle 4th	29th to 32d
Middle 5th	33d to 36th

TABLE 2.—*Weight, Crown-Rump and Crown-Heel Length During Fetal Life*

Lunar Month	Length: Crown-Rump: Streeter*	Length: Crown-Rump: Scammon and Calkins†	Length: Crown-Heel: Dietrich‡	Length: Crown-Heel: Scammon and Calkins†	Weight: Streeter*	Weight: Scammon and Calkins†
Second	2.3	....	3.0	....	1.1	....
Third	7.4	5.17	9.8	7.08	14.2	24.0
Fourth	11.6	10.76	18.0	15.54	108.0	70.0
Fifth	16.4	15.54	25.0	22.79	316.0	230.0
Sixth	20.8	19.77	31.5	29.20	630.0	620.0
Seventh	24.7	23.63	37.1	35.05	1045.0	1250.0
Eighth	28.3	27.18	42.5	40.43	1680.0	1600.0
Ninth	32.1	30.51	47.0	45.47	2478.0	2300.0
Tenth	36.2	33.63	50.0	50.20	3405.0	2950.0

* Streeter, G. L.: Contrib. Embryol. **11**: 145, 1920.
† Scammon and Calkins.[3]
‡ Dietrich, H. A., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1924, vol. 6, p. 177.

TABLE 3.—*Degree of Maturity of Infants as Defined in the Chicago Lying-In Hospital*

Abortive group
1. Length less than 28 cm.
2. Weight less than 400 Gm.
3. Gestation less than 22 weeks

Premature Group
A. Previable
1. Length from 28 to 34.9 cm.
2. Weight from 400 to 999 Gm.
3. Gestation from 22 through 27 weeks
B. Viable
1. Length from 35 to 46.9 cm.
2. Weight from 1,000 to 2,499 Gm.
3. Gestation from 28 through 36 weeks

Group at term
1. Length from 47 to 54 cm.
2. Weight from 2,500 to 4,500 Gm.
3. Gestation from 37 to 43 weeks

Postmature group
1. Length more than 54 cm.
2. Weight more than 4,500 Gm.
3. Gestation more than 43 weeks

For purposes of classification the fetus or infant is placed in the group with the criteria of which two or more of its measurements are found to correspond

TABLE 4.—*Organ Weights in Grams* *

Organ	Male	Female
Brain	407	391
Heart	23.24	21.37
Lungs	60.24	56.10
Liver	158.56	162.70
Spleen	10.73	10.73
Pancreas	3.7	3.7
Adrenals	9.75	9.02
Kidneys	27.63	24.75
Thyroid	1.9	2.2
Thymus	11.64	10.54
Placenta	652	645

* The figures are the median weights of organs for 163 male and 153 female infants at the end of the tenth month of gestation. For the male the median weight and length were 3,181 Gm. and 52.3 cm., and for the female, 3,018 Gm. and 50.7 cm.

internal organ to show evidence of degeneration is the liver, which undergoes autolysis very quickly. The nuclei disintegrate and the tissues lose the ability to stain normally about the same time as the skin changes are found. Very soon serosanguineous pleural and peritoneal effusions occur, all organs undergo progressive softening and acquire a uniform color similar to that of the fluid in the cavities. In the early stages after death all tissues and fluids are bright red. As the red blood cells disintegrate the color changes to a dull opaque violet, which persists for from several days to a few weeks. This gradually fades, so that if the dead fetus remains within the uterus for from six to eight weeks almost all color is lost and the entire body is light grayish yellow. Calcium may be deposited in the tissues, as it is in any dead tissue within the body, and a lithopedion may result.

The organs within the abdomen lose their cellular structure before those of the thoracic cavity. Lung tissue may stain sufficiently well to show its general structure many days after death has occurred, and the existence within the alveoli of hemorrhage, pus cells, cells from the amniotic fluid and other material may be easily demonstrated. The muscles are the last structures to lose cellular pattern. Cartilage, although retaining its general form, changes from a clear translucent white to red early in the process of maceration.

Maceration is diagnostic evidence of nothing except that the fetus died in utero. Syphilis is but one cause of intra-uterine death, although the possibility that it is the cause should be investigated in every fetus. Frequently there are no gross abnormalities, and any microscopic changes are obliterated by the maceration so that demonstration of spirochetes in the tissues is the only way of making a diagnosis of syphilis certain. These organisms are found most constantly in the liver and the adrenal, and if absent there, are probably not demonstrable in any of the tissues. In advanced maceration the spirochetes, too, are destroyed.

Skin and Subcutaneous Tissues.—The color of the skin of a non-macerated infant may vary normally from white to deep violet. The darker color is due partly to antenatal engorgement of peripheral vessels and partly to postmortem stasis. Icterus at birth or within the first day or two is uncommon and when present is frequently associated with erythroblastosis. Later in the neonatal period it is physiologic and of no pathologic significance. The jaundice resulting from congenital obstruction of the bile ducts does not usually develop until several weeks after birth.

Marked extensive edema is rarely present except in association with erythroblastosis. It may involve the entire trunk, head and extremities, or any portion may escape. When the tissues are incised, the muscle layers are found widely separated by a homogeneous jelly-like substance, from which fluid is easily expressed. Localized areas of edema, usually

associated with hemorrhage or extreme congestion, are frequently found on the portion of the fetus which presented at the pelvic inlet during delivery. They are most common on the scalp or the buttocks. In breech presentations the scrotum or labia frequently show marked edema and hyperemia.

Lacerations and contusions may result from injury incurred during birth (especially as marks from forceps blades) or they may be signs of criminal violence. The type and location usually make the one class easily separable from the other.

Eruptions are extremely uncommon in the new-born infant, although either macular or papular lesions may occur in syphilis. Other associated syphilitic lesions which may be present at birth or which may develop within a few days are bullae of the palms and soles and rhagades around the mouth, nose or anus. Pemphigus neonatorum is an intradermal infection, and its extent may vary from a few lesions a few millimeters in diameter to areas involving most of the cutaneous surface. In the latter case it usually results in generalized septicemia which leads to death. Inflammatory areas of varying extent and severity may occur about the umbilicus, the more severe occasionally resulting in septicemia. Bacteria gain entrance through the stump of the umbilical cord and spread through contiguous structures.

Developmental defects of the skin may occur in various locations, the most common being in the midline near the vertex of the skull. Those on the head are usually from 1 to 2 cm. in diameter and ordinarily involve only the skin. If the infant survives, the area is usually epithelialized without treatment. More extensive skin defects are usually associated with other malformations.

Sclerema, a more or less generalized induration of the skin produced by an abnormality of the fat cells; scleroderma, an involvement of the skin also produced by abnormality of fat cells and characterized by multiple minute indurated areas, and scleredema (von Reuss[5]), a condition in which there is localized abnormality of fat cells plus edema of the affected part, giving a characteristic doughy consistency to the tissues, are all rare. They are also difficult to diagnose after death because the high melting point of normal fat in the infant causes marked generalized hardening of the tissues when the body is cooled.

Icthyosis is a rare congenital condition in which the cutaneous surface is composed of yellow, white or grayish smooth horny scales or plaques. Death is due to interference with the normal functions of the skin.

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5. von Reuss, A.: Diseases of the Newborn, London, J. Bale, Sons & Danielsson, 1920.

Muscles.—There is usually little of pathologic significance demonstrable in the muscles. Rigor rarely occurs in a stillborn infant or one dying soon after birth and is not marked in one which has lived only a few days. Injury of muscles during birth is uncommon except injury of the sternocleidomastoid muscle, and even this does not ordinarily occur unless the muscle is originally abnormally short or hypoplastic.

Bony Skeleton.—Malformations involving the skeleton are fairly common. Defects or absence of the bones of the cranial vault or of the posterior portion of the spinal column are the most common of all major congenital malformations. They are frequently found in the same infant and almost invariably are associated with abnormalities of the underlying nervous tissues.

Other abnormalities include the absence of or, less commonly, the duplication of any or all bones of the extremities, clefts or fissures especially involving the bones of the face (e. g., the maxilla or the palate) or an abnormal relation of parts without actual malformation of the bone substance (e. g., clubfeet, clubhands and scoliosis of the spine).

Generalized abnormalities of the skeleton are present in osteogenesis imperfecta and chondrodystrophia foetalis. The former is characterized by abnormal fragility of bones, which is due to thinness of the cortex, and is evidenced by numerous intra-uterine or extra-uterine fractures. The fractures heal readily with a large amount of callus formation. The ribs at birth occasionally have a beaded appearance, due to a succession of healed fractures. The diaphyses of the long bones are usually slender and often irregular in shape; the epiphysial lines are normal. The skull is poorly ossified, and the cranial vault is usually composed of numerous thin, small, irregular islands of bone separated by membrane. The disease is a primary disturbance in the formation of osseous tissue.

Chondrodystrophia foetalis is characterized by thickening and shortening of the bones, with some irregularity of the epiphysial lines. The head is usually large and the trunk well developed, but the extremities are unusually short. It is a primary disturbance in cartilaginous growth. Malacic, hypoplastic and hyperplastic types have been described.

Fractures may be produced in normal bones during delivery of the infant. They are most common in the long bones and in the clavicle. Occasionally the skull may be fractured by improper application of the forceps, but there is usually sufficiently little calcification of the cranial bones to permit bending instead of fracturing. Separation of the alar and basal portion of the occipital bone may cause marked injury to brain tissue through encroachment of the separated portions on the cranial cavity. The separation is usually produced by forcing

the posterior portion of the head against the symphysis pubis in an attempt to save the perineum during delivery.

The belief that rickets may occur as an intra-uterine condition has both opponents and proponents, although most recent writers tend to deny that it occurs. A soft skull, gaping fontanels and wide sutures are sometimes interpreted as intra-uterine rickets but are probably merely signs of delayed ossification and bear no specific relation to the rickets of infancy and early childhood. The original name for chondrodystrophia foetalis was fetal rickets.

Congenital syphilis evidences itself most frequently as a change in the epiphysial line of the long bones. The demarcation between bone and cartilage is normally a sharp even line, but in syphilis the line is irregular, thickened and yellowish. Microscopically, irregularity of the line of ossification and fibrosis of the terminal portion of the metaphysis are characteristic. Roentgenograms of the long bones show a thickened opaque epiphysial line.

Cranial Vault, Brain and Spinal Cord.—The head is the most important part of the body in determining whether or not death was due to trauma sustained at birth. Many infants, even in the absence of other signs of trauma, exhibit a caput succedaneum. This is a serosanguineous accumulation of fluid in the tissues between the scalp and the pericranium on the presenting portion of the head. It may be palpated as a boggy mass under the scalp and is in itself never a cause of death. A somewhat more severe injury is the cephalhematoma, or hemorrhage under the pericranium of one or more bones of the cranial vault, usually the parietal bones. It is limited by the margins of the bone and if on the external surface will usually resorb leaving no permanent damage. If it is on the internal surface and if the hemorrhage is sufficient, it may produce an increase in intracranial pressure such that death may result.

The head is best opened by Benecke's technic, which consists in cutting large windows on the lateral aspects of the head (fig. 1). The scissors are inserted low in the occipitoparietal suture, and an incision is made which is extended upward almost to the posterior fontanel. It is then extended anteriorly, cutting through the upper portion of the occipital bone just below the superior sagittal suture in such a way as to miss the attachment of the falx cerebri. It is carried downward again along the frontoparietal suture. This makes possible sufficient examination of the brain and cranial vault while leaving an attachment at the base which insures the embalmer adequate support in restoring the head. The incision is made through both bone and dura.

The condition of the falx and tentorium and the relation of hemorrhage to them are among the most important points to investigate in the

newly born infant. If the skull cap is removed in the manner common to necropsies on adults, these relations are disturbed and the structures are partially destroyed. If the body of the infant is not to be formally buried but is to be disposed of by the hospital, a more accurate examination of the head and its contents is possible, after fixation in solution of formaldehyde U. S. P. In such a case the flaps on the side of the head may be entirely removed, and the head may be severed from the trunk and placed in the solution of formaldehyde for several days

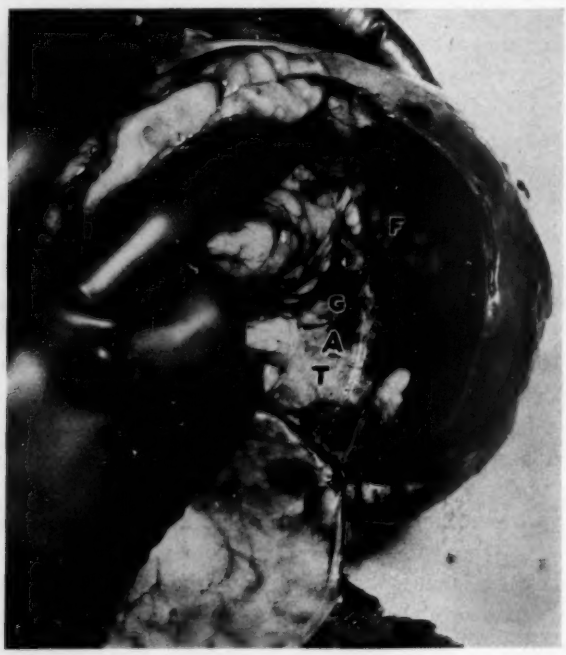

Fig. 1.—Normal head opened by the Benecke technic. The left cerebral hemisphere has been drawn forward to expose the falx cerebri (*F*) and the tentorium cerebelli (*T*). Three branches of the internal cerebral vein are visible. The internal cerebral vein (vein of Galen, indicated by *G*), the total length of which is less than 1 cm., may be seen entering the straight sinus at the junction of the falx and tentorium. The point *A* on the tentorium cerebelli is the usual location of tentorial lacerations.

before the brain is removed. The brain is normally extremely soft, and much difficulty is experienced in attempting to remove it in the fresh state without injury to the substance. A preliminary examination of the falx and tentorium should be made before fixation, as it will be necessary to cut through these structures to remove the hardened brain. Better preservation of tissues will be obtained if the fixing fluid is

injected into the brain through the internal carotid arteries before the brain is immersed in it.

The brain in the fresh state is examined through the openings in the parietal bones, the side not under inspection being supported and protected by keeping the opposite flap closed. The color of the brain is the same as in the adult, but the consistency is much less firm. The leptomeninges are delicate structures and, particularly in the premature infant, the subarachnoid space frequently contains considerable fluid so that the cerebral blood vessels are raised from the surface of the brain. Slight extravasation of red blood cells into the meninges may be unassociated with trauma. Conditions, such as severe anoxemia, which cause changes in capillary pressure may give rise to extravasation of this type. In the absence of other hemorrhage or sign of injury, this is not sufficient to warrant a diagnosis of intracranial hemorrhage. This diagnosis, if placed on a death certificate, automatically puts the death statistically in the group of deaths from birth traumas. It is possible to hold theoretically that slight hemorrhage over the surface is an indication of a trauma occurring at birth in which nerve cells were sufficiently injured to cause death even in the absence of gross or histologic changes, but the diagnosis on the protocol of the autopsy should rest on anatomic proof and not on theory. Slight superficial intrameningeal hemorrhage is in itself not enough to cause death. When there is free blood over the cerebrum, the problem is entirely different, for this finding occurs only after rupture of vessels, and this is known to occur only from injury.

After the surface of the cerebrum has been examined, the occipital lobe may be gently drawn forward and the tentorium cerebelli, together with its attachment to the falx cerebri, inspected. Extravasation of blood cells into the leaves of either of these supporting structures is common and of little significance as regards the cause of death. Sudden or excessive alteration in the shape of the head puts an abnormal strain on both falx and tentorium and may cause laceration of the falx or of one or both sides of the tentorium. The inferior sagittal sinus courses through the free margin of the falx, and injury to the falx leads to hemorrhage from the sinus. The tentorium is much more commonly lacerated than the falx, and unless the laceration is sufficiently deep to extend into the straight sinus, there is no hemorrhage from the laceration itself. The laceration is commonly associated, however, with rupture of the internal cerebral veins (veins of Galen) or of the great cerebral vein at the point where it enters the straight sinus. Bleeding is usually into the subtentorial space. Since this is a closely confined area, relatively little blood may cause sufficient increase in pressure to produce death through injury to the vital centers in the medulla and pons.

The substance of the brain infrequently contains gross hemorrhage but may do so even in the absence of bleeding in other portions of the cranial vault. Sections for microscopic examination are preferably taken from the floor of the fourth ventricle. Hemorrhage in the pons or medulla is fairly definite evidence of injury to vital centers.

The premature infant, owing to the greater fragility of its tissues, reputedly incurs a greater risk of injury to the brain during delivery than does the infant at term. The greater fragility of tissues is probably more than compensated by the smaller size of the head and the lack of marked molding during passage through the birth canal. At the Chicago Lying-in Hospital, intracranial hemorrhage is slightly less common in premature infants than in infants born at term.

Intradural hemorrhage in the spinal canal is usually associated with subtentorial bleeding. It is due to direct transmission of blood cells through the spinal fluid. Occasionally the spinal column may be injured so as to give rise to local hemorrhage. (The sixth or seventh cervical vertebrae are most commonly traumatized.) The spinal cord may be injured either by formation of a hematoma or by pressure from displaced vertebrae. The spinal cord can be easily exposed by cutting through the laminae of the vertebrae with heavy scissors or a fine rib cutter after the skin and muscles have been reflected.

Inspection of the spinal canal is too frequently omitted by the average pathologist. Unless there is a specific reason why the spinal canal cannot be opened, examination of it should invariably be included as part of the necropsy. The statement that the cause of death could not be determined is never justifiable if the interior of the vertebral column has not been investigated.

Face.—The eyes in the newly born infant are uncommonly abnormal. If the infant has lived for a few days there may be inflammation and discharge. If this is profuse and purulent, it may be due to a neisserian infection incurred at the time of delivery, and smears of the pus should be stained for gonococci. A slight, more watery secretion which develops immediately may be due to a reaction to a silver preparation instilled at the time of birth. The cornea becomes opaque a short time after death, especially if the lids are not closed. This process should not be confused with congenital cataract.

The nose in the newly born infant rarely displays any pathologic change except that associated with harelip or other facial defect. Occasionally in congenital syphilis rhagades may be present around the nares and mouth.

The mouth and larynx should be inspected for edema, malformation, a foreign body or other obstruction to the air passages. The tongue is occasionally enlarged and may protrude through the open mouth. This occurs most commonly in mongolism and erythroblastosis. A cleft

in either or both the maxillary process and hard palate is the most frequent malformation.

The extent of development of the ears is of some value as an aid in determining the degree of maturity of the fetus. It is only as the fetus approaches term that cartilage develops in the pinna. Before this time the ears are soft and relatively shapeless. Malformation of both the internal and external ear occurs occasionally. A newly born infant is rarely subject to inflammation of the ears, but older infants may acquire meningitis as a result of extension of a middle ear infection into the mastoid region and into the venous sinuses.

Body Cavities.—Exposure of the abdominal and thoracic cavities is obtained in the same manner as in the adult (fig. 2). Scissors are used to make a midline incision from the symphysis pubis to a point near the center of the sternum. The incision is then carried laterally on each side to the upper part of the shoulder, a V-shaped flap being formed at the base of the neck. The skin is reflected back over the wall of the chest, and the peritoneum is cut along the costal margin to expose the abdominal cavity.

The peritoneal cavity is examined before the thoracic plate is removed. The position, relative size and external appearance of each organ, the absence or presence of blood or other fluid in the cavity and if possible the point of origin are noted. More detailed examination is better accomplished after the thoracic organs have been removed. The diaphragm is inspected and the height of the dome recorded. Defects of the diaphragm are much more common on the left than on the right side and so-called pseudohernia (simple absence of a portion of the diaphragm) is more common than true diaphragmatic hernia (defect in the musculature but with a thin membrane present). The stomach is the organ which abdominal pressure most commonly forces into the thoracic cavity. With a small defect, only a portion of the stomach may be found in the thoracic cavity, but more frequently, in dead newly born infants, the diaphragmatic hernia contains most of the abdominal viscera except the liver and the retroperitoneal organs. The heart is pushed to the right, and the lungs, particularly the left, are often imperfectly developed. Microscopically, the bronchi are large and prominent, while the alveoli are few, small and collapsed, as well as abnormally immature in their degree of development.

The anterior wall of the chest is removed by cutting through the ribs lateral to the cartilaginous portion, severing the diaphragmatic attachment and cutting the costoclavicular junctions. The neck is exposed by dissecting the triangular flap of skin upward.

Thyroid Gland.—The thyroid gland lies at about the level of the thyroid cartilage and presents a broad isthmus with two plump lateral

lobes which encircle the trachea for about two thirds of its circumference. The gland is occasionally hypertrophied and more rarely absent. Occasionally in an infant born of a myxedematous mother who is not receiving treatment the gland is enlarged, and the infant becomes cretinous if treatment is not instituted. Absence of the thyroid also produces cretinism and is the usual cause in the sporadic cases of this condition in which the mother is apparently normal.

Fig. 2.—Normal viscera of a newly born infant at forty weeks' gestation. Note especially the size and location of the thymus and liver. Only a small area of lung is visible, the major portions lying behind the heart. *T* indicates the thyroid; *Tr*, the trachea; *Th*, the thymus; *Ht*, the heart; *Lu*, the lung; *L*, the liver; *St*, the stomach; *SI*, the small intestine; *Sp*, the splenic flexure of the colon; *Si*, a portion of the sigmoid colon.

Tumors, particularly teratomas, may be located in the region of the thyroid gland. Fistulas, cysts or tumors in this region are usually associated with improper closure of the branchial clefts. The second cleft is the one most frequently involved.

Parathyroid Glands.—The parathyroid glands are rarely visible grossly in the newly born infant. Almost invariably what seems like parathyroid tissue at autopsy proves to be accessory thymic tissue on microscopic examination. Not uncommonly, however, on microscopic examination of the thyroid gland a parathyroid gland is found embedded in the substance or immediately under the capsule. It is rarely more than 1 mm. in diameter. The importance of the function of the parathyroid gland in the fetus and immediately after birth is unknown. No congenital tumor of such a gland has been reported. Wyatt and McEachern[6] reported a case of congenital dysplasia of bone in which the parathyroid glands measured from 1.5 to 3 mm. in diameter, and they believed that this hyperplasia was related etiologically to the condition. The fact that tetany of the new-born may be relieved by administering parathyroid substance indicates that even at this early period calcium metabolism and the function of the parathyroid glands are closely associated.

Thymus.—The thymus is composed of a broad portion, which lies over and partially encircles the anterior superior surface of the pericardium and great vessels, and two small tonguelike projections extending a short distance into the neck. Occasionally accessory nodules of thymic tissue are found higher in the neck, and frequently they are attached to the thyroid. The weight of the thymus in the new-born varies fairly consistently with the weight of the organs in general, although occasionally it is unusually small or great. The diagnosis of an enlarged thymus or of status thymicolymphaticus as the cause of death is made too frequently on insufficient evidence. Roentgenograms are unsatisfactory in establishing a diagnosis of an enlarged thymus. What is generally called the thymic shadow probably includes a shadow made by the great vessels. It frequently varies in size, depending on whether the infant is quiet or crying. Occasionally an abnormal shadow due to a congenital cardiac abnormality may be interpreted as representing an enlarged thymus. In the neonatal period the cause of death cannot invariably be determined at autopsy, and it is better to admit this than to obscure the facts by a hypothetical diagnosis.

Round, sharply circumscribed hemorrhages, about 1 mm. in diameter, are frequently found on the surface and in the parenchyma of the thymus when interference with circulation through the umbilical cord has occurred in utero. Microscopically, the hemorrhages are visible as isolated areas of red blood cells in the cortex of the gland. The microscopic structure of the thymus is very constant during the entire development of the fetus and shows little change from the end of the third to the tenth month.

6. Wyatt, T. C., and McEachern, T. H.: Am. J. Dis. Child. **43**:403, 1932.

Heart.—After the thymus has been carefully dissected away from the pericardium and vessels with which it is in intimate contact, the heart lies exposed. In a macerated fetus and frequently in an infant with fetal hydrops, the pericardial cavity contains excessive fluid. Hemorrhage within the sac is extremely infrequent. However, in one case at the Chicago Lying-in Hospital an eroded area was present on the anterior surface of the right ventricle which had given rise to hemorrhage sufficient to distend the pericardial sac. On microscopic examination the area revealed hemorrhage into the myocardium and superficial infiltration with polymorphonuclear leukocytes. Small subepicardial hemorrhages are frequently associated with similar hemorrhages in the thymus and lungs. They are most frequent along the anterior and posterior longitudinal coronary sulci.

The heart in the newly born infant occupies a somewhat more vertical position than does the heart in the adult. With the exception of the right atrium, it is usually contracted after death. The right atrium is frequently dilated, especially in infants stillborn as a result of intra-uterine asphyxia.

The principal differences in the heart of the new-born as compared with that of the adult are in the presence of the ductus arteriosus and foramen ovale. The ductus arteriosus is a vessel the caliber of which approximates that of the aorta or pulmonary artery and which in the fetus forms the channel permitting the major portion of the blood entering the right atrium to enter the aorta (fig. 3). The right and left pulmonary arteries are given off the main pulmonary trunk approximately 1 cm. beyond the margin of the pulmonary valve. The pulmonary trunk continues for another centimeter and empties into the arch of the aorta distal to the orifice of the left subclavian artery. This portion between the origin of the two pulmonary arteries and the aorta is the ductus arteriosus (fig. 3). When the vessels are opened so as to expose the intima, a distinct difference is visible. The ductus arteriosus presents a finely wrinkled semitranslucent surface while the aorta and the pulmonary artery are smooth and opaque. After a few days the ductus arteriosus begins to show irregular patches of intimal thickening, the musculature contracts, and the entire circumference of the vessel becomes less. The closure is a gradual process, and at three months or more a minute lumen may remain.

The foramen ovale, which is a normal opening in the interatrial septum during fetal life, becomes functionally closed with the establishment of respiration. It remains anatomically patent, however, for varying lengths of time, occasionally even throughout adult life. At autopsy in the neonatal period it is difficult to prove that the foramen remained patent after birth. If the foramen was functionally patent,

there are usually an associated hypertrophy of the pulmonary artery and ductus arteriosus and hypoplasia of the aorta.

If thorough examination of the heart and great vessels in situ shows them to be entirely normal, the heart may properly be removed by severing the inferior and superior vena cava, pulmonary veins, aorta and pulmonary artery. If any abnormality is discovered, a more satisfactory specimen may be obtained by removing the heart, ductus arteriosus, aorta and lungs without severing the individual structures. The heart is opened for inspection of the interior by following the path of circulation of the blood. With the posterior surface upward, the scissors

Fig. 3.—Normal heart of a newly born infant at forty weeks' gestation. The heart is drawn to the right to expose the ductus arteriosus and to show the relation of this vessel to the other vessels. Note that the caliber of the ductus arteriosus is approximately that of the aorta and of the main pulmonary artery. *A* indicates the arch of the aorta; *DA*, the descending aorta; *D*, the ductus arteriosus; *P*, the main pulmonary artery; LP, the branch of the pulmonary artery to the left lung; *LL*, the left lung; *RV*, the right ventricle; *RA*, the right auricular appendage.

are inserted into the opening of the inferior vena cava in the right atrium, an incision is made which extends downward through the tricuspid valve and along the right side of the interventricular septum to the apex. The heart is then turned over and the incision continued along the interventricular wall until finally the pulmonary valve has been cut.

The left side of the heart is opened in a similar way, starting with the opening of one of the pulmonary veins and ending at the aortic valve.

The cardiac valves are very fine and delicate in structure. In the fetus at term the aortic valve has a slightly greater circumference than the pulmonic valve, and the tricuspid valve, a slightly greater one than the mitral valve. Hematomas a fraction of a millimeter in diameter may be found on the margins of the valves, especially on the atrial surface of the mitral or of the tricuspid valve. They result when small clefts normally present on the valve surface become engorged with blood and they occur most frequently in association with marked visceral congestion.

Malformations of the heart are almost never a cause of stillbirth and may or may not cause early death of a new-born infant. Minor malformations are frequently compatible with life, and it is only the more marked ones that cause death in the first few days. The most common malformations causing early death are (1) atresia or marked stenosis of the pulmonary artery, usually associated with transposition of the arterial vessels, and (2) a persistent atrioventricular ostium. There are frequently other associated abnormalities, a defect in the ventricular septum being especially common with transposition of the vessels.

Trachea and Lungs.—After the heart has been removed, the trachea and main bronchi are dissected free from surrounding tissues. They are then opened and examined for blood, mucus or other fluid or solid obstruction. If a tracheal catheter has been used in an attempt to institute respiration, the examination of the pharynx and trachea should be especially thorough.

The most common malformation of the trachea is a tracheo-esophageal fistula. The usual type is one in which the upper portion of the esophagus ends blindly a short distance below the pharynx, while the lower portion originates from an opening in the trachea at its bifurcation.

The pleural cavities rarely contain fluid except in macerated infants and those exhibiting generalized fetal hydrops. In the former the fluid is serosanguineous; in the latter, a clear amber color. Petechial hemorrhages may occur beneath the parietal pleura under the same conditions which produce them in the thymus and lungs.

The lungs are the organs most constantly abnormal in newly born infants, and if microscopic studies were to be made of only one tissue in the body this would prove far the most valuable. In stillborn infants the lungs are completely atelectatic unless expanded by use of a tracheal catheter, by mouth to mouth breathing or by some other form of artificial respiration. It may be difficult on gross examination to distinguish normal from artificial expansion. Expansion of the lungs in the live-born infant is a gradual process. On the first inspiration a few alveoli

are opened, and on succeeding breaths the number is increased and the size augmented. Von Reuss [5] stated that the lung capacity is 17 cc. twenty minutes after birth, 21 cc. three and a half hours after birth and 36 cc. six hours after birth. The depth of respiration increases considerably between the first and second day, but many days pass before complete expansion occurs. Thus partial atelectasis is physiologic in young infants. Any factor which inhibits normal respiration decreases the rate of expansion. Atelectasis, although it may be the immediate cause of death, is never the primary one. It is merely a symptom of inability of the infant to carry on a normal process. This may be due to abnormality of the central nervous system as in (1) malformation of the brain, (2) pressure on the respiratory center caused by subtentorial hemorrhage, (3) immaturity of the cells of the respiratory center, (4) injury of cells of the respiratory center by drugs or as a result of an inadequate supply of oxygen; or it may be due to a local difficulty in the lungs as in (1) immaturity of the parenchyma, with insufficient alveolar development to permit the normal procedure of gaseous metabolism, (2) hypoplasia of lung tissue due to a defect of the germ plasm, (3) aspiration of amniotic fluid, blood or mucus, producing blockage of localized areas and (4) intra-uterine pneumonia with plugging of alveoli by polymorphonuclear leukocytes; or it may be due to indirect causes such as (1) pressure on the lungs from distention of the abdomen by polycystic kidneys, intestinal distention or other conditions and (2) pressure from viscera herniated into the pleural cavity through a diaphragmatic defect. Occasionally an infant, although at term, does not have sufficient ability to use its thoracic muscles and diaphragm normally. There are instances in which at necropsy no pathologic condition other than a greater or lesser degree of atelectasis can be found, but even in such cases the cause is nondemonstrable, not absent. Atelectasis when given as a cause of death usually means "cause of death undetermined."

Marked congestion of lung capillaries is very frequently observed in stillborn infants and infants dying in the first few days of life. When it is extreme, the capillaries rupture and bleeding into the alveoli and under the visceral pleura ensues. Subpleural hemorrhage is usually petechial and is uncommon except in rather abrupt anoxemia. Premature separation of the placenta produces the most striking number of such hemorrhages. Occasionally in the bodies of infants several days old congestion and hemorrhage in the lungs are the only abnormalities found. Cruickshank [7] classes such cases as instances of fulminating

7. Cruickshank, J. N.: The Causes of Neonatal Death, Medical Research Council, Special Report Series, no. 145, London, His Majesty's Stationery Office, 1932.

pneumonia in which the infection was so overwhelming that there was not time for polymorphonuclear cell invasion. Others believe that this is one manifestation of a hemorrhagic diathesis which kills the infant before other organs are involved. Further investigation is needed before a definite decision can be made.

Pneumonia may be present in stillborn or live-born infants. It is rarely either distinctly bronchial or lobar in distribution but is usually a diffuse involvement of irregularly scattered alveoli. If it is intrauterine in origin, squamous cells from inspired amniotic fluid are frequently associated with the polymorphonuclear luekocytes in the alveoli. Little or no fibrin is present, and areas of hemorrhage may be present or absent. In young infants a gross diagnosis of pneumonia is very difficult to make, for congestion and hemorrhage of the lungs, especially when these occur in partially atelectatic areas, may produce the same appearance. Sections of the lungs should be examined microscopically in all cases in which pneumonia is suspected, before a positive diagnosis is made. In fact, a positive statement as to the presence or absence of pneumonia in a stillborn or a newly born infant can rarely be made except on microscopic examination.

Poor expansion of the lungs has long been considered an etiologic factor in the production of infantile pneumonia. However, in view of the frequency with which pneumonia is present in stillborn infants, the infiltration of the alveoli by leukocytes may at least occasionally be the cause of the apparent atelectasis rather than the result.

The lungs of many infants who die in utero contain varying amounts of amniotic fluid. This is manifested by the presence of material in the alveoli which has its origin from the body of the fetus. With hematoxylin-eosin stain vernix caseosa appears as a pink homogeneous substance adherent to and forming a partial lining of the alveolar walls. Squamous epithelial cells are usually blue-violet and are occasionally seen from the flat surface, but more frequently each cell is a long, irregular, compressed mass. Meconium may be occasionally present within macrophages in alveoli or septums.

Malformation of the lungs rarely occurs except as the production of anomalous lobes or as arrested development. Hypoplasia of the lungs may occur independently but is more commonly associated with other malformations. In a diaphragmatic hernia of the left side, with many of the abdominal viscera in the left pleural cavity, the left lung may be a small, rudimentary structure and weigh only a few grams. Microscopically, the bronchi are large and prominent and the alveoli poorly developed.

Gastro-Intestinal Tract.—In the newly born infant the intestine is longer in relation to the size of the body cavity than it is in the older child or adult, so the bowel, especially the large intestine, lies in more

redundant loops. At birth, unless meconium has been passed into the amniotic sac, the large bowel is commonly distended with green-black, extremely viscid material. This is composed of mucus, bile, intestinal secretions, fat globules, cholesterol crystals, vernix caseosa, epithelial cells and hairs.

Farber [8] suggested a simple method for determining, even without autopsy, whether or not a congenital intestinal obstruction is present. If squamous epithelial cells are present in a stained smear of meconium, no obstruction exists, for their source is swallowed amniotic fluid.

Congenital atresia is the most common malformation of the gastro-intestinal tract and most frequently involves the esophagus, the rectum or the part of the intestine where the yolk sac was originally attached.

Congenital peritonitis is usually the result of overdistention of a portion of the intestinal tract due to atresia, herniation through a defect in the mesentery or other similar condition. There may be actual rupture of a viscus with production of sterile peritonitis through the chemical irritation of meconium, or a portion of the intestine may become necrotic, as through strangulation of a hernia, and peritonitis result without rupture of a viscus. Rupture of the bowel has been reported as occurring during delivery, but this is almost impossible unless there is some factor causing the preceding distention.

Bleeding from the intestinal tract (melena) is of relatively frequent occurrence. The source of blood has occasionally been found in ulcerations of the duodenum and pyloric end of the stomach. Some investigators believe such bleeding to be due to a hemorrhagic diathesis characterized by a prolonged bleeding and clotting time. This condition is due probably to lack of prothrombin in the blood and is characterized clinically by hemorrhage from mucous membranes.

Air in the intestine has been considered of medicolegal significance. Hajkis [9] stated that in artificial introduction of air into the esophagus (by mouth to mouth breathing, for instance) it will not pass beyond the pyloric sphincter and that consequently air in the intestine is indicative of an intra-uterine existence. At the Chicago Lying-in Hospital this does not hold true, for my associates and I have seen large quantities of air in the intestines of infants who have never breathed. Air in the intestine does mean, however, either that the infant has breathed or that air has been artificially introduced.

In older infants gastro-enteritis is of frequent occurrence, and in those between 1 month and 1 year of age it is one of the more common causes of death. Infants rarely present such a condition within the first two weeks of life. However, epidemics of infectious diarrhea of the new-born do occur, with a high incidence of deaths.

8. Farber, S.: J. A. M. A. **100**:1753, 1933.
9. Hajkis, M.: Lancet **2**:134, 1934.

Liver and Bile Ducts.—The liver during fetal life undergoes more change in both gross and microscopic appearance than any other organ in the body except the lungs. During the last few weeks of gestation it gains remarkably in size. Microscopically, as the fetus approaches full development, the areas of erythropoiesis, so numerous in early fetal life, gradually disappear, and at full term few remain. In erythroblastosis, especially when associated with generalized edema, the areas of developing erythroblasts persist, and the liver resembles that of a fetus in a much earlier stage of development. The liver is grossly enlarged, and a marked increase in nucleated red blood cells in all stages of development occurs in the circulating blood. (See table 5 for normal figures for blood cells at birth.)

TABLE 5.—*Cellular Constituents of the Blood of Infants in the Neonatal Period*

	Lucas and Others*			Lippman†	
	At Birth	5 Days	12 Days	At Birth	5 Days
Hemoglobin, per cent	117	107	91		
Erythrocytes	5,511,000	4,983,000	4,533,000	5,200,000	5,400,000
Leukocytes	19,200	10,900	13,200	16,600	9,500
Differential count					
Polymorphonuclear leukocytes	70	49	30	45.4	30.8
Eosinophilic leukocytes	1	3	2	1.8	2.0
Transitional and mononuclear cells	9	15	14	2.0	2.0
Lymphocytes	20	34	48	44.6	63.0
Nucleated red cells per 100 leukocytes	..	..	..	1.6	0
Platelets	305,000	295,000	..	213,000	
Bleeding time, minutes	1½-3	..	..	1½-2½‡	
Coagulation time, minutes	15	..	..	2-4‡	

* Lucas, W. P.; Dearing, B. F.; Hoobler, H. R.; Cox, A., and Jones, M. R.: Am. J. Dis. Child. **22**: 525, 1921.
† Lippman, H. S.: Am. J. Dis. Child. **27**: 473, 1924.
‡ These figures are taken from Merritt and Davidson (Am. J. Dis. Child. **46**: 990, 1933).

Icterus gravis has recently been considered one manifestation of erythroblastosis. In this condition the fetus either is born with icterus of a high grade or presents it shortly after birth. The amniotic fluid and placenta are also occasionally yellow. Death usually occurs within the first few days of life. The diagnosis may be made ante mortem by the increased number of nucleated red blood cells in the circulating blood. When jaundice and erythroblastosis are associated, the microscopic observations are practically the same as in fetal hydrops. Severe early icterus is, however, not invariably associated with erythroblastosis. Necrosis of the liver is occasionally the only pathologic change visible. The term "icterus gravis" at present means little more than severe icterus of unknown etiology. If abnormalities in the formation of red blood cells are present, the condition should be called by the more specific term "erythroblastosis."

The liver undergoes rapid autolysis after death, and in advanced maceration it is frequently markedly softened. The staining characteristics of the tissues are lost within a few hours after intra-uterine death.

Marked congestion and distention of sinusoids are frequently present when death has been due to interference with placental circulation. A subcapsular hematoma is usually the result of injury at birth and if ruptured may cause death from hemorrhage.

Fatty metamorphosis of liver cells occurs with unusual rapidity in infants suffering from acute infection of any type, the degree depending on the duration and severity of the infectious process.

Syphilis may produce marked changes in the liver, the most characteristic being intralobular cirrhosis. Congenital syphilis is almost the only condition producing an involvement of this type. Erythropoiesis frequently persists longer than usual.

Malformation of the liver is not common, although modification of its shape occurs in any condition which alters the contour of the chest or of the abdomen. Multiple cysts of the liver are frequently found associated with polycystic kidneys. Atresia of the bile ducts results in biliary cirrhosis; in fact, the most typical pure biliary cirrhosis is found in young infants with this condition. The bile ducts normally have narrow lumens and are frequently too small to admit a probe. The best way to determine patency is to expose the papilla of Vater in the duodenum. Bile can easily be expressed from it by pressure on the gallbladder if the cystic and the common duct are patent. Cross-sections of the ducts examined microscopically will settle the problem if there is any doubt.

Pancreas.—The pancreas rarely presents gross abnormalities of any kind. Microscopically, the islands of Langerhans vary in size and appearance with the stage of development of the fetus. The amount of insular tissue is reputedly increased when maternal diabetes is present, and hypoglycemia resulting from oversecretion of insulin has been suggested as a cause of early death in live-born infants of diabetic mothers. This is extremely difficult to prove. At this hospital very numerous giant islands have been seen in infants born of nondiabetic mothers, and foci of lymphocytes, also reputedly characteristic of diabetes, have been found frequently in the head of the pancreas in normal new-born infants. An increase in connective tissue may be present in congenital syphilis. Foci of primitive blood cells may occur in erythroblastosis.

Spleen.—The spleen rarely shows gross change other than variation in size and the presence of accessory nodules of splenic tissue. There may be one or several nodules a few millimeters in diameter. They are entirely separate from the spleen and are usually found near the lower or near the medial border. In syphilis the spleen may be moderately enlarged as a result of general hyperplasia and infiltration by hemosiderin-filled macrophages, and in erythroblastosis it may be more markedly enlarged as a result of abnormal hematopoiesis.

Kidneys, Ureters and Bladder.—The kidneys frequently show marked congestion, and pinpoint-sized red areas may be present on the surface. These represent minute subcapsular hemorrhages. Microscopically, hemorrhage may also be present at the apex of the pyramids. Hemorrhages of both types are most commonly present when there is extreme congestion due to interference with placental circulation. Uric acid infarcts, described as small yellow or yellow-brown concretions in the parenchyma, were reported as of frequent occurrence in the kidneys of newly born infants by the early investigators. I have found them very infrequently.

Malformations of ureters or kidneys are observed occasionally, but the majority of them are not incompatible with life. Double ureter, hydro-ureter, abnormalities of form or of location of either or both of the kidneys are found only incidentally, death being due to other conditions. Bilateral agenesis of the kidney causes death almost immediately after birth, and the process in polycystic kidneys may, if marked, result in death at or shortly after birth. The cysts are usually small but numerous, and the kidneys may reach an immense size (in a case at the Chicago Lying-in Hospital, the combined weight of the kidneys was 250 Gm., the infant weighing 2,670 Gm.). I have observed multiple cysts in the kidneys of a fetus 20 cm. long. The condition has been described as due to failure of fusion of secretory and collecting tubules, and more recently as persistence of cysts which normally occur in the very young fetus as a result of the degeneration of the first three or four generations of nephrons to be found in the permanent kidney. Neither explanation is completely satisfactory.

Microscopically, the kidney in the immature fetus presents a peripheral margin of developing tubules and glomeruli called the nephrogenous zone. In the fetus at term this zone has practically disappeared, and by its presence or absence and the number of layers of glomeruli in the cortex the degree of maturity may be fairly well estimated.

The urine of normal infants in the first few days of life commonly contains a small amount of albumin and occasionally casts, leukocytes, epithelial cells and red blood cells.

The bladder is rarely abnormal except as a result of malformation. Extroversion, the commonest anomaly, may be found alone or in combination with other malformations. When present as the only abnormality, it produces death in the neonatal period very infrequently.

Adrenals.—The adrenals are larger in comparison with the size in the adult than any other organ in the fetus except the thymus. Frequently, numerous minute nodules of accessory adrenal tissue are present around the periphery of the gland.

In the new-born infant the adrenal structure varies markedly from that present later in life. The peripheral portion is narrow and is com-

posed of irregular dark-staining masses of basophilic cells, from which the definitive cortex is eventually derived. The central portion is several times greater in width and is made up of large acidophilic cells separated by wide sinusoidal channels. This portion disappears within the first few weeks after birth. True medullary cells in the new-born infant are inconspicuous. Owing to the width of the sinusoidal spaces between the acidophilic cells, the gland is frequently engorged with blood and on casual microscopic examination may appear hemorrhagic without being so. The central portion degenerates very quickly after death, becoming red-brown and almost fluid, and a gross diagnosis of hemorrhage into the adrenal is consequently too frequently made.

Occasionally, massive hemorrhage into the adrenal occurs so that a gland weighing normally from 3 to 4 Gm. may weigh 35 Gm. or more. (I recently observed two cases in one of which the hemorrhage was unilateral, with an adrenal weight of 35 Gm.; in the other the hemorrhage was bilateral, with adrenal weights of 20 to 25 Gm.) Adrenal hemorrhage is found most frequently in infants born by breech presentation and is believed to be usually due to abdominal injury inflicted during delivery of the aftercoming head. Death may be due either to hemorrhage or to acute adrenal insufficiency.

The adrenals are characteristically markedly hypoplastic in anencephalic monsters. They are occasionally absent, but if careful search is made miniature glands weighing from 0.1 to 0.2 Gm. are usually found.

Genital Organs.—Male: The testes usually descend about the sixth month of intra-uterine life, although either one or both may not be in the scrotum at birth. Rarely a hydrocele or a hematocele may be present. The most common malformation is a cleft in the penis. When it is on the upper surface, the condition is known as epispadias; when on the under surface, as hypospadias.

Female: The cervix in the new-born infant is very much larger in proportion to the corpus than is that in the adult and usually comprises about two thirds of the entire uterus. The vagina has a thick covering of squamous epithelium, which varies in thickness in different areas, giving the vagina a rugose appearance. The cervix occasionally contains cysts measuring up to 1 cm. in diameter. They are usually under the squamous epithelium and are unrelated to the cervical glands. The ovaries frequently contain small developing follicles, and occasionally follicular cysts of considerable size are present. The cysts are filled with either clear fluid or blood. A cyst from an infant who was stillborn at term, containing 10 cc. of fluid, was observed which on biologic assay was found to contain estrogen in an amount comparable to that in the cystic follicle of the adult. Such cysts are believed to be a response

to maternal endocrine secretions which have entered the fetal circulation by way of the placenta.

Malformations in the genital system are fairly common. They are practically never the cause of death. True hermaphroditism, in which functioning gonads of both sexes are present in one person, is almost unknown in the human species. Pseudohermaphroditism, in which the external genital organs simulate those of the sex opposite to that typified in the gonads present or exhibit characteristics of both sexes, is not infrequent.

In the female pseudohermaphrodite hypertrophy of the clitoris, penetration of the clitoris by the urethra or adherence of the labia to simulate a scrotum may be present individually or in any combination. In the male pseudohermaphrodite the most common form is hypospadias peniscrotalis with a more or less rudimentary development of the penis. It may be impossible to distinguish the cleft scrotum from labia and it may be impossible to determine the sex positively from external examination.

Placenta.—The placenta presents many variations which, although of interest anatomically, have little known relation to fetal death. The main changes of importance to the pathologist are those which interfere with circulation to such an extent that the fetus does not receive adequate oxygen and food material.

The most common placental cause of fetal death is premature separation. A retroplacental blood clot of varying size may be formed and an excavation in the placenta under it is frequently produced. The villi are compressed, and circulation through the fetal vessels in this area is prevented. Grossly the placenta in this region is firm and pale, with the consistency of an early infarct.

Placenta praevia ranks next in importance as a cause of fetal death. Death rarely occurs until after the onset of labor, and if elective cesarean section is the treatment of the condition and there are no other complicating factors a live infant will usually be secured. In delivery from below, death of the fetus may occur from severe maternal hemorrhage. If a Braxton Hicks' version is done or if a bag is inserted to control hemorrhage, it may be necessary to penetrate the placenta to get within the uterine cavity. In such a case both fetal and maternal blood vessels are torn, and death may be due either to fetal hemorrhage or to anoxemia. Even in the absence of marked hemorrhage the infant frequently succumbs owing to anoxemia following a Braxton Hicks' version.

Marked infarction of the placenta may limit the functioning area to such an extent that there is insufficient blood space for transfer of fetal and maternal metabolites. Little is known of the cause. Both infarction of the placenta and death of the fetus are fairly commonly found in

severe maternal toxemia. Fetal death may occur in toxemia without infarction of the placenta and vice versa, but when death occurs in the presence of massive infarction it is possible that infarction was the primary result of toxemia and fetal death secondary.

Adair and Thelander [10] found the average fetal-placental weight relationship about 1 : 7.27. It is reasonable to believe that placental growth may fail to parallel fetal growth and that the nutritive requirements of the fetus may exceed the placenta's ability to furnish them. How small the placenta may be in relation to the fetus and still normally maintain life has never been determined.

Evidence of acute infection in the placenta is uncommon and as a cause of fetal death is difficult to prove. It is seen occasionally when membranes have been ruptured a considerable length of time before the onset of labor or when labor is prolonged. Even if bacteriologic studies show the same organism in the placenta and fetus, it may be concluded that the source of the infection in both is the same and that one is not necessarily the precursor of the second.

Syphilis and tuberculosis both produce pathologic changes in the placenta although either disease may be transmitted from the mother to the fetus without production of lesions in the placenta. In syphilis, when the placenta is involved, its size and weight are frequently increased. Microscopically, clubbing of terminal villi and increase in the size of individual villi, due to abnormal connective tissue and cellular content, are produced. The only way of making a positive diagnosis is by demonstrating spirochetes in the tissues.

Jeans and Cooke [11] in 1921 observed 389 infants 2 months of age in whose cases the placenta had been examined and the Wassermann test had been made on blood from the umbilical cord at the time of birth. In this group there were 22 infants with syphilis (positive Wassermann reaction). In only 14 of these cases (63.6 per cent) had the Wassermann reaction of the blood from the umbilical cord been positive, and in only 6 (27 per cent) had histologic changes in the placenta been found. Of over 2,000 infants in whose cases the Wassermann test on blood from the umbilical cord and placental examination had been made, not one having a negative result from the Wassermann test or a normal placenta was found to have syphilis at the end of two months.

Tuberculosis of the placenta is extremely rare, but tubercles in both decidua and placenta have been described.

Erythroblastosis, especially that associated with fetal hydrops, may produce a marked increase in the size of the placenta. The villi are large, and frequently knoblike projections are visible. Numerous

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11. Jeans, P. C., and Cooke, J. V.: Am. J. Dis. Child. **22**:402, 1921.

nucleated red cells are present in fetal vessels and in foci in the villi outside the vessels. The diagnosis of erythroblastosis may occasionally be made from the placenta alone when the fetus has died in utero and the organs have become too degenerated for microscopic examination.

SUMMARY

The postmortem examination of the stillborn infant and the infant dying in the neonatal period presents certain differences from postmortem examination of the adult. The causes of death in the stillborn and the liveborn infant are in general similar and are largely related to antepartum and intrapartum obstetric complications. There is a small group of infants who succumb because abnormalities of the ova or spermatozoa have prevented normal development and there is a small group who die from infections. Most of those remaining die from abnormal factors associated with the placenta, umbilical cord, birth processes or the establishment of extra-uterine life.

To determine the cause of death is frequently difficult, and if the body of the infant is the only material available it is frequently impossible. To make it possible to arrive at a reliable conclusion, an adequate clinical history must be available, and the placenta, cord and infant must be examined. Autopsy of the infant should invariably include examination of all parts of the body, including the cranial, thoracic and abdominal cavities, the neck, the vertebral column and the long bones. Microscopic examination of the viscera is essential.

EXPERIMENTAL ATHEROSCLEROSIS

I. EFFECT OF PROLONGED ADMINISTRATION OF THE THYROTROPIC FACTOR OF THE ANTERIOR LOBE OF THE PITUITARY ON EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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Since atherosclerosis was first produced experimentally in rabbits by the feeding of cholesterol about twenty-five years ago (Anitschkow;[1] and Wacker and Hueck[2]), an appreciable amount of work dealing with the pathologic phases of this problem has been carried out both in this country and abroad. Recent studies have centered more or less on the prevention or inhibition of experimental atherosclerosis by a variety of agents; obviously, such investigations have promise of clinical application. One of the most important steps in the study of experimental atherosclerosis in rabbits was the observation made by Murata and Kataoka[3] in 1917 and later adequately confirmed by Liebig,[4] Seel and Creuzberg,[5] Turner,[6] Friedland[7] and Page and Bernhard[8] that the feeding of iodine prevented the deposition of this lipid in the arterial walls. Moreover, Turner and Khayat[9] showed that thyroidectomy abolishes this protective action of potassium iodide. A striking relationship between experimental atherosclerosis and the function of the thyroid gland was thus established.

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5. Seel, H., and Creuzberg, G.: Arch. f. exper. Path. u. Pharmakol. **161**:674, 1931.
6. Turner, K. B.: J. Exper. Med. **58**:115, 1933.
7. Friedland, I. B.: Ztschr. f. d. ges. exper. Med. **87**:683, 1933.
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In view of these findings, it appeared desirable to determine what effect the pituitary gland, through its elaboration of the thyrotropic factor, exerts on experimental atherosclerosis in rabbits. The present report details our studies on this subject.

MATERIAL AND METHODS

Twenty-one rabbits varying in age from 3 to 5 months were obtained. Five were set aside as controls and observed for from 103 to 105 days (group A). Three rabbits received an injection of 0.5 cc. of thyrotropic factor daily six times a week for from 108 to 114 days (group B). Four were given an injection of 1 cc. of 0.9 per cent sodium chloride intraperitoneally every day six days a week for from 101 to 124 days; to the diet of each was added 1 Gm. of cholesterol three times a week (group C). Three rabbits received 0.25 cc. of thyrotropic factor daily six times a week for from 118 to 120 days; this group also received 1 Gm. of cholesterol three times a week (group D). Three were given 1 cc. of thyrotropic factor twice a week for from 115 to 117 days; the same amount of cholesterol was also added to their diets (group E). Three rabbits were given an injection of 1 cc. of thyrotropic factor daily six times a week for from 103 to 107 days; in this group, cholesterol was also added to the diet (group F).

The basic diet was a rabbit chow sold commercially,[10] which was fed to the rabbits daily. Water was left in the cages at all times. Cholesterol, when fed, was mixed with the daily rations.

The thyrotropic factor of the anterior lobe of the pituitary used was prepared under the supervision of Collip and obtained through Ayerst, McKenna and Harrison, of Montreal; this extract was always injected intraperitoneally. It is reported to contain only insignificant amounts of the other anterior pituitary factors. A preliminary assay of the extract carried out in this laboratory according to the method described by Loeb and Friedman[11] revealed that it had marked thyrotropic activity. Four guinea-pigs of almost identical weight were obtained. Two were kept as controls; two received 2 cc. of the thyrotropic factor intraperitoneally daily for 3 days. On the fourth day all the guinea-pigs were killed. The thyroids were excised and weighed. Those of the controls weighed 41 and 41.4 mg., respectively; those of the animals which had received the thyrotropic factor weighed 52.6 and 91.6 mg., respectively.

At the end of the experimental period, each rabbit was killed instantaneously by fracturing the cervical spine, immediately opened, and the whole length of the aorta, including approximately 1 inch (2.5 cm.) of both common iliac branches, removed. The vessel was then incised longitudinally, dried to a constant weight in a low temperature oven maintained at 60 C., mixed with fat-free sand, macerated in a mortar and the cholesterol extracted by two washings with boiling 95 per cent alcohol and one with ether. The combined alcohol-ether extracts were made up to a known volume and the total cholesterol and ester cholesterol determined

10. This product is prepared by the Ralston Purina Company, St. Louis, and contains a mixture of grains and alfalfa hay, supplemented by vitamins and minerals. This chow is reported to contain iodized salt, but the amount of iodine present is presumably very small since the chow in no way interfered with the deposition of cholesterol in the arterial tree of any control animal fed cholesterol.

11. Loeb, L., and Friedman, H.: Proc. Soc. Exper. Biol. & Med. **29**:14, 1931.

on two aliquots by the Bloor-Knudson procedure [12] with control of temperature as practiced in this laboratory.[13] The cholesterol values were expressed as grams per hundred grams of dry weight of aorta.

The whole blood cholesterol was determined in each rabbit every 15 to 17 days during the period of observation. The method of Schoenheimer and Sperry [14] as modified for the colorimeter by Fitz [15] was employed. The results of this investigation will be described in a later communication. The average value of the whole blood cholesterol referred to in this report represents an average of all the values determined during the experimental period.

The Cholesterol Contents of the Aortas of Control Rabbits (Group A), of Rabbits Receiving Thyrotropic Factor Alone (Group B), of Rabbits Receiving Cholesterol Alone (Group C) and of Rabbits Receiving Both Cholesterol and Thyrotropic Factor (Groups D, E and F)

Rabbit	Duration of Experiment, Days	Cholesterol Content of Aorta, Gm. per 100 Gm. of Dry Weight				Comment
		Total	Ester	Free	Ester/Free Ratio	
Group A	...	....	....	....	....	Control
1	103	0.36	0.13	0.23	0.58	
2	135	0.36	0.16	0.20	0.79	
3	135	0.75	0.17	0.58	0.29	
4	106	0.46	0.18	0.28	0.85	
5	106					
Group B	...	....	....	....	....	0.5 cc. thyrotropic factor daily for 6 days a week
1	108	0.36	0.16	0.20	0.81	
2	114	0.50	0.32	0.18	1.76	
3	114	0.92	0.41	0.51	0.79	
Group C	...	....	....	....	....	1 Gm. cholesterol 3 times a week; 1 cc. of 0.9% NaCl daily for 6 days a week
1	123	0.35	0.15	0.20	0.73	
2	123	3.23	0.97	2.26	0.47	
3	124	3.28	1.47	1.81	0.81	
4	101	1.63	0.84	0.79	1.07	
Group D	...	....	....	....	....	1 Gm. cholesterol 3 times a week; 0.25 cc. thyrotropic factor daily for 6 days a week
1	118	5.98	4.71	1.27	3.72	
2	118	1.64	1.02	0.62	1.63	
3	120	2.12	1.16	0.96	1.20	
Group E	...	....	....	....	....	1 Gm. cholesterol 3 times a week; 1 cc. thyrotropic factor twice a week
1	115	4.99				
2	115	2.00	1.00	1.00	1.00	
3	117	0.88	0.44	0.44	1.00	
Group F	...	....	....	....	....	1 Gm. cholesterol 3 times a week; 1 cc. thyrotropic factor daily for 6 days a week
1	103	5.08	3.36	1.72	1.95	
2	107					
3	107	4.90	3.20	1.70	1.89	

RESULTS

The accompanying table details the cholesterol contents of the aortas of all the animals used in this study. This protocol shows that the administration of thyrotropic factor alone over a protracted period of time exerts little influence on the cholesterol content of the aorta (group B). The administration of cholesterol and physiologic solution of sodium chloride is associated with a marked increase in the cholesterol

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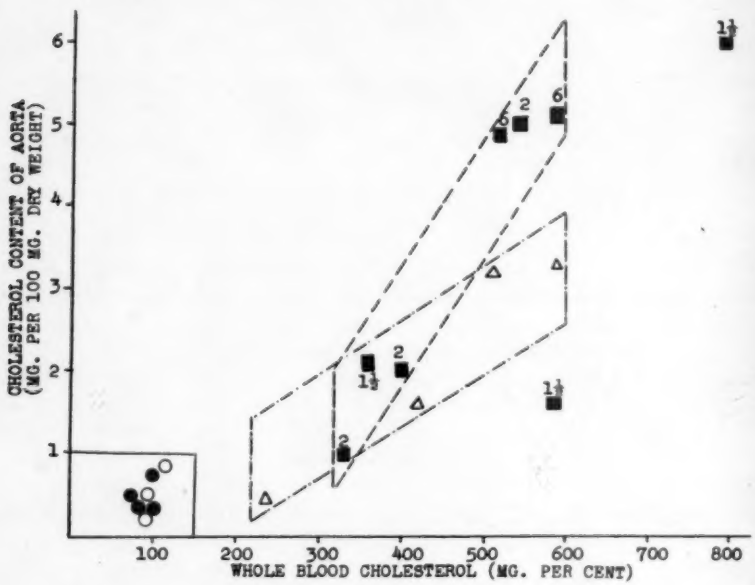
14. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.
15. Fitz, F.: J. Biol. Chem. **109**:523, 1935.

content of this vessel, and, for the most part, there seems to be little difference in the ratio of ester to free cholesterol in the aorta in which atherosclerosis has been produced thus when compared with controls (group C). However, if cholesterol and thyrotropic factor are simultaneously administered to rabbits over a prolonged period, there appears to be in many instances a greater deposition of cholesterol in the aorta (this was also confirmed macroscopically). Again, the ratio of ester to free cholesterol frequently becomes elevated (groups D, E and F).

The accompanying figure demonstrates the relation of the average value of the whole blood cholesterol to that of the cholesterol in the



Relation of the average value of the whole blood cholesterol to that of the cholesterol of the aorta in control rabbits, in rabbits receiving thyrotropic factor alone, in rabbits receiving cholesterol and physiologic solution of sodium chloride and in rabbits receiving both cholesterol and thyrotropic factor. Solid circles represent controls; hollow circles, rabbits receiving thyrotropic factor alone (3 cc. per week); hollow triangles, rabbits receiving 3 Gm. of cholesterol per week and 1 cc. of physiologic solution of sodium chloride daily; solid squares, rabbits receiving 3 Gm. of cholesterol per week and the designated quantity of thyrotropic factor per week.

aorta in the control and experimental animals. When all the animals are studied as a single group, the cholesterol content of the aorta appears to vary directly with the average concentration of cholesterol in the blood. The figure further demonstrates that rabbits receiving thyro-

tropic factor plus cholesterol tend to show a somewhat disproportionate increase in the cholesterol content of the aorta when compared with the animals receiving cholesterol without thyrotropic factor even though the average values of the whole blood cholesterol in both groups are approximately the same.

COMMENT

When these studies were first planned, it was felt that the increased thyroid activity produced by the injection of thyrotropic factor might exert a definite influence on the degree of experimental atherosclerosis in rabbits. It was felt that the administration of this substance might inhibit the deposition of cholesterol in the arterial tree in the same manner as does potassium iodide, thyroxin and thyroid substance. Our findings, however, show that the administration of thyrotropic factor to rabbits not only does not inhibit the deposition of cholesterol in the arterial tree but tends to increase it. There appears, however, to be a good reason why this occurs.

Collip and Anderson [16] demonstrated that the administration of thyrotropic factor to animals over a prolonged period is first accompanied by an increase in the consumption of oxygen but later the rate of the metabolism becomes depressed owing to the formation of an antithyrotropic substance. Turner [17] has shown that rabbits repeatedly given injections of thyrotropic factor first show a rise in oxygen consumption, then, in spite of continued injections, the consumption of oxygen falls and returns to the preinjection level and in some cases decreases considerably on the minus side. Although our facilities did not permit metabolic studies in our rabbits, it may be assumed that the administration of this factor over a period of more than 100 days was probably accompanied by depression of the rate of the metabolism in these animals, particularly in the latter part of the experimental period.

Rabbits metabolize cholesterol poorly since this substance is foreign to their diet, but it is probable that when their rate of body metabolism is depressed their ability to store or excrete the ingested cholesterol is impaired. The clinical counterpart may be observed in the hypercholesteremia of hypothyroidism. Again, in view of what has been stated at the outset of this paper, one must agree with Leary [18] that aberrations of thyroid function appear to be factors in the deposition of cholesterol in the arterial tree. Thus, two potent factors, namely, (1) increased difficulty in metabolizing cholesterol and (2) vascular susceptibility to deposition of cholesterol, probably favored the excessive deposition of cholesterol in the aorta in animals receiving thyrotropic factor.

16. Collip, J. B., and Anderson, E. M.: Lancet **1**:76, 1934.

17. Turner, K. B.: Personal communication to the authors.

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In a careful study of 123 healthy persons who had died suddenly from violence, Landé and Sperry [19] showed that the concentration of cholesterol in the blood serum is not related to the degree of atherosclerosis in the aorta as measured by its lipid content. Similarly, Duff,[20] in a recent review of the literature on experimental atherosclerosis, concluded that hypercholesteremia is not solely responsible for the development of the atherosclerotic lesions in the aorta of the rabbit fed cholesterol. Our results, however, demonstrate, in confirmation of those reported by Versé,[21] that the degree of experimental atherosclerosis is dependent on the hypercholesteremia.

In a study of human autopsy material, Zeek [22] recently demonstrated a marked increase in the ratio of free to ester cholesterol in the advanced atherosclerotic lesions of the aorta; her findings confirmed those of Meeker and Jobling.[23] Our results demonstrate that rabbits fed free cholesterol and receiving thyrotropic factor simultaneously frequently show a disproportionate increase in the ratio of ester to free cholesterol (a similar increase was originally observed by Schoenheimer [24] for human atherosclerosis, but, as indicated in the foregoing sentence, were not confirmed by Meeker and Jobling or by Zeek).

CONCLUSIONS

The intraperitoneal administration of the thyrotropic substance of the anterior lobe of the pituitary over a protracted period (more than 100 days) to rabbits fed cholesterol is accompanied in most instances by an increase in the deposition of cholesterol in their aortas as compared with the aortas of control animals receiving cholesterol alone. This increase in the cholesterol content of the aorta is frequently associated with an augmentation of the ratio of ester to free cholesterol. Rabbits receiving the thyrotropic substance alone show no change in the total cholesterol content of the aorta or in the ratio of ester to free cholesterol when compared with controls. The extent of atherosclerosis, as gaged by the cholesterol content of the aorta, appears to vary directly with the degree of hypercholesteremia. In an attempt to explain these findings, some theoretical considerations are discussed.

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COMPARATIVE BACTERIOLOGIC STUDIES OF HUMAN BLOOD, VISCERA AND TEETH OBTAINED AT NECROPSIES

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That bacteria are present in the periapical areas of 43 per cent of noncarious teeth has been shown in a previous study.[1] The proportion of apexes involved was found to increase in the presence of caries or restorations of the crowns. Streptococci were isolated from 89 per cent of the teeth with positive periapical cultures. Other kinds of organisms, such as Staphylococcus aureus, Staphylococcus albus, Bacillus coli and Diplococcus-pneumoniae, were isolated less frequently from periapical tissues but usually in association with Streptococcus. The pathway of these organisms into the periapical tissues of the teeth with caries and restorations may well be through the pulp, but in the intact, noncarious teeth invasion must be either by way of the peridental membrane or by the blood stream.

In the present work, therefore, an attempt has been made to determine from data obtained post mortem the role played by bacteremia in the localization of bacteria at the apexes of the teeth.

METHODS

The general significance of the bacteriologic observations after death and the methods employed in this study have been described fully in previous reports.[2] The same varieties of medium were employed for cultures from blood, teeth and viscera. Both aerobic and anaerobic methods were used in all of the cultural procedures.

Ninety-three persons from whom 195 periapical dental cultures were procured post mortem had had bacteriologic examinations of the blood during life or at necropsy or both before and after death. Of these, thirty-eight gave positive blood cultures either clinically or at the time of postmortem examination, and fifty-five gave negative blood cultures, although several clinical cultures had been obtained from many of the latter persons during their illness.

This article is a contribution from the Dental Study Unit.

From the Department of Pathology, School of Medicine, Yale University.

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RESULTS

The incidence of growth from the blood, viscera and periapical tissues of the teeth is given in table 1.

Positive growth was obtained in cultures from blood, viscera and teeth in practically identical proportions.

However, there was observed a discrepancy between the kinds of organisms present in these three different sites (table 2).

TABLE 1.—*Incidence of Bacterial Growth in Postmortem Cultures*

Source of Cultures	Observations	With Growth		Without Growth	
		Number	Percentage	Number	Percentage
Blood (93 necropsies)	93	38	40	55	60
Organs—(liver, spleen, right and left kidneys)—(64 necropsies)	249	111	45	138	55
Periapical areas of teeth (93 necropsies)	195	91	47	104	53

TABLE 2.—*Distribution of Types of Organisms*

Kind of Organism	Frequency			
	In Periapical Tissues of Teeth with Growth (91 Observations)		In Blood Stream and Viscera with Growth (149 Observations)	
	Number	Percentage	Number	Percentage
Streptococcus viridans	89	97	44	29
Streptococcus nonhaemolyticus	25	27	8	6
Streptococcus haemolyticus	16	17	33	23
Diplococcus pneumoniae	6	7	40	26
Staphylococcus aureus and Staph. albus	44	48	63	42
Bacillus coli	8	9	63	42
Diphtheroids	1	..	6	4
Green-producing bacillus	1	..	1*	..
Anaerobic gram-negative gas-forming bacillus	1	..	1*	..
Unidentified gram-negative bacillus	5	5	9	6
Pseudomonas pyocyanea	0	..	2	1
Bacillus lactis-aerogenes	0	..	1	..
Yeast	0	..	3	2
Clostridium Welchii	0	..	36	24
Bacillus alcaligenes	0	..	6	4
Proteus vulgaris	0	..	6	4
Haemophilus influenzae	0	..	9	6
Gram-negative cocci	4	4	0	..
Micrococcus tetragenus	1	..	0	..
Anaerobic gram-positive bacillus	2	..	0	..
Meningococcus	0	..	1*	..

* Only the clinical culture was positive.

The bacteria isolated from the periapical tissues of the teeth were those belonging to the normal flora of the mouth, including strains of Streptococcus viridans, Streptococcus nonhaemolyticus, Staph. aureus or albus, the diphtheroids and gram-negative cocci. In addition, hemolytic streptococci, pneumococci, colon bacilli and other pathogens were isolated, but less frequently.

The incidence of each type of organism that occurred both in the periapical tissues and in the blood stream of the same person is given in table 3.

Among the thirty-eight cases in which the blood stream was recognized as infected there were twenty-one (55 per cent) in which the apexes of the teeth yielded an organism similar morphologically and culturally to that obtained from the blood stream. In seventeen of these twenty-one cases bacteriologic examination of the oral cavities showed the presence of organisms similar to those isolated from the blood stream and from the tissues around the teeth.

From the same twenty-one cases there were thirty-one (58 per cent) teeth the apexes of which, respectively, yielded an organism similar to the one found in the blood stream. One tooth only was cultured in eight of these cases while in the remainder from two to six teeth were examined bacteriologically.

TABLE 3.—*Incidence of Types of Organisms Found in Both Blood and Teeth*

Kind of Organism	Blood Cultures Positive (38 Observations)	Blood Cultures Showing Same Bacterium as Teeth Showed		Apexes of Teeth Giving Positive Cultures (106 Observations)	Apexes Showing Same Bacterium as Blood Showed
		Number	Percentage		
Streptococcus viridans	8	4	50	27	5
Streptococcus nonhaemolyticus	1	..	0	5	0
Streptococcus haemolyticus	8	3	37	24	5
Diplococcus pneumoniae	10	2	20	25	2
Staphylococcus aureus or Staph. albus	7	6	85	15	8
Bacillus coli	7	3	43	23	8
Diphtheroids	1	..	0	4	0
Green-producing bacillus	1*	1	100	5	1
Anaerobic gram-negative gas forming bacillus	1*	1	100	5	1
Unidentified gram-negative bacillus	1	1	100	2	1
Haemophilus influenzae	1	..	0	4	0
Clostridium Welchii	1	..	0	1	0
Meningococcus	1*	..	0	1	0

* A clinical blood culture was positive.

In one of the necropsies B. coli was isolated from the blood and apical tissues of all six teeth examined bacteriologically.

Of the thirty-one teeth that gave positive cultures of the same kind of organism as was found in the blood stream, ten (32 per cent) were normal, noncarious teeth while twenty-one (68 per cent) had defects in their crowns consisting of caries, widely exposed pulp canals, fillings and restorations, and three of these teeth consisted only of roots. The fact that there were gross pathologic changes in the crowns of the teeth suggests that in some of the cases the organisms seen in the cultures from the periapical tissues might have gained entrance to these tissues by way of the pulp canal instead of by way of the blood stream.

Further analysis of the data concerning the ten intact, noncarious teeth suggest that in five of them the infections might have been the result of invasion through the damaged soft tissues of the oral cavity or through the mucous membranes of an infected maxillary sinus.

Extensive necrotizing gingivitis was found at necropsy in a case of agranulocytic angina and in one of ammonia poisoning. In the former Str. haemolyticus was isolated from the periapical tissues of three teeth, the oral cavity and the blood stream. A strain of Pneumococcus was cultured from one of the two teeth from the person who had extensive ammonia burns of the gingiva, as well as from the oral cavity and the blood stream. An infected maxillary sinus of another person gave at necropsy a growth of B. coli and Staph. aureus identical with the periapical culture of the tooth adjacent to the infected sinus. Histologically, the remaining periapical tissues of this tooth showed no anatomic evidence of infection.

In summary, eleven of the thirty-one apexes of the teeth which gave positive cultures may be considered to have been infected by way of the blood stream. None of these eleven apexes showed histologic evidence of an acute or chronic inflammatory process.

Evidence of dissemination from an infected dental focus is suggested by the fact that transient bacteremia occurred in two persons ten days before death. The organisms isolated from these persons were sufficiently distinctive both morphologically and culturally to be readily identified when isolated from the tissues post mortem. A single blood culture containing an unidentified gram-positive bacillus forming green pigment was obtained from a patient with mitral endocarditis ten days before death. Subsequent clinical blood cultures yielded Str. viridans and once Str. haemolyticus. Post mortem one of five teeth yielded Str. viridans and a gram-positive green-forming bacillus similar to the one obtained in the clinical culture. The remaining four teeth were free from growth, although both Str. haemolyticus and Str. nonhaemolyticus were isolated from the blood and viscera. The bacteriologically positive tooth showed a large diffuse chronic inflammatory process. The clinical blood culture from the other patient showed transient bacteremia from an unidentified gram-negative gas-producing bacillus, also obtained ten days before death. Subsequent blood cultures, made clinically and at postmortem examination, as well as the visceral cultures, were free from bacteria. One of three teeth cultured yielded a gram-negative gas-producing bacillus identical with the one isolated clinically.

In eight of fifty-five cases in which both the clinical and the postmortem blood cultures were negative Str. haemolyticus and D. pneumoniae were isolated from the periapical tissues of teeth. However, these organisms were also recovered from the viscera and oral cavity in six of the cases. One necropsy even showed both hemolytic streptococci and pneumococci at the apexes and in the visceral tissue. These findings suggest that unrecognized transient bacteremia may have occurred during life to account for the positive periapical cultures, but

repeated clinical blood cultures in four of the cases failed to show the presence of these organisms in the blood stream.

The possibility of unrecognized bacteremia occurring clinically is suggested by the fact that at necropsies a high incidence of pathogenic organisms was found in the viscera of persons whose clinical and postmortem blood cultures were negative. There were forty-four persons in whom necropsies revealed visceral infection, as shown by bacteriologic examinations of the liver, spleen and both kidneys, making a total of 111 visceral and 84 periapical dental cultures.

Table 4 gives the incidence of the various kinds of bacteria in cultures from the viscera and teeth in the forty-four necropsies.

The difference in kinds of organisms isolated from the viscera and teeth of the same person is apparent from these data. Pathogenic organ-

TABLE 4.—*The Various Kinds of Bacteria Isolated at Forty-Four Necropsies*

Kind of Bacteria	Necropsies in Which Positive Cultures Were Obtained (44 Observations)		Frequency with Which Organs (Liver, Spleen and Kidneys) Gave Positive Cultures (111 Observations)		Frequency with Which Apical Tissues Gave Positive Cultures (84 Observations)	
	Number	Percentage	Number	Percentage	Number	Percentage
Streptococcus viridans	10	22	12	10	62	74
Streptococcus nonhaemolyticus	3	7	4	3	20	23
Streptococcus haemolyticus	3	7	5	4	11	13
Diplococcus pneumoniae	2	4	4	3	4	5
Staphylococcus aureus or Staph. albus	16	36	27	24	27	32
Diphtheroids	5	11	5	4	1	1
Bacillus coli	21	45	37	34	0	..
Clostridium Welchii	10	22	28	25	0	..
Haemophilus influenzae	3	7	5	4	0	..
Proteus vulgaris	2	4	6	5	0	..
Bacillus alcaligenes	2	4	6	5	0	..
Pseudomonas pyocyanea	1	2	2	2	0	..
Bacillus lactis-aerogenes	1	2	1	1	0	..
Yeast	1	2	2	2	0	..

isms, such as Str. haemolyticus, D. pneumoniae, Haemophilus influenzae, B. coli and others, were isolated from the viscera without any blood cultures being recognized as positive either clinically or at postmortem examination.

Sufficient data are not available pertaining to the significance of the duration of bacteremia in the localization of organisms at the apexes of teeth. In fifteen cases in which the clinical blood cultures were positive, nine of these positive cultures were procured twenty-four hours before death; one, on the fourth day; one, on the fifth; one on the sixth day, and the remaining two cultures were obtained one month and two months before death.

The relation of a previously existing chronic inflammatory reaction at the apexes of the teeth to the localization of bacteria from the blood stream is only suggestive. Of thirteen teeth with histologic changes at the apexes revealed in necropsies at which the blood cultures were found

positive, seven (23 per cent) yielded, respectively, an organism similar to the one obtained in the blood stream. On the other hand, of seventy-four periapical areas without lesions, twelve (16 per cent) showed the presence of organisms of the same type as those found in the blood stream.

COMMENT

These observations indicate that at least 10 per cent of the teeth cultured in necropsies on persons known to have had bacteremia will show an organism morphologically and culturally similar to the one recovered from the blood stream. The cultural methods which we employed to distinguish the different strains were not satisfactory, particularly when Str. viridans, Staph. aureus or Staph. albus was the predominant organism isolated from the teeth. These cultural methods are undoubtedly of more significance in the identification of the less frequently isolated pathogenic strains, such as Str. haemolyticus, D. pneumoniae, B. coli and a few other pathogens. The finding of the more pathogenic strains of organisms in cultures from the apexes of the teeth in the presence or absence of bacteremia at several of the necropsies suggests the importance of the blood stream as a portal of entry into dental periapical tissues. On the other hand, the significance of bacteremia in accounting for the high incidence of Str. viridans and Staphylococcus at the apexes of the teeth is difficult to demonstrate unless it is possible to show that there are repeated transitory invasions of the blood stream by these organisms during life.

Transitory bacteremia has been recognized to occur in both healthy and chronically ill persons. Cameron, Rae and Murphy [3] and Reith and Squier [4] obtained positive blood cultures from healthy persons in from 6 to 12 per cent of the cases examined. In cases of chronic illness the frequency of positive blood cultures increases, particularly in those cases in which there is a localized lesion related to infection. Cameron, Rae and Murphy [3] obtained positive blood cultures in 27 per cent of cases of chronic periapical infections of the teeth. Cecil, Nicholls and Stainsby [5] demonstrated as high as 60 per cent positive cultures from persons with infectious arthritis. McEwen, Alexander and Bunim [6] demonstrated bacteremia in 24 per cent of arthritic patients. Minor operative

3. Cameron, G. C.; Rae, C. A., and Murphy, G. M.: Canad. M. A. J. **25**:31, 1931.

4. Reith, A. F., and Squier, T. L.: J. Infect. Dis. **51**:331, 1932.

5. Cecil, R. L.; Nicholls, E. E., and Stainsby, W. J.: Arch. Int. Med. **43**: 571, 1929.

6. McEwen, C.; Alexander, R. C., and Bunim, J. J.: J. Lab. & Clin. Med. **21**:453, 1936.

procedures, such as extraction of teeth (O'Kell and Elliott [7] Burket and Burn [8]) and tonsillectomy (Fischer and Gottdenker [9]), also have been shown to permit transitory invasion of the blood stream by bacteria. Even a physiologic process, for example, menstruation, has been shown by Kulka [10] to give rise to bacteremia. Changes in diet will cause transitory invasion of the blood stream by bacteria, as shown by Desoubry and Porcher,[11] Nedzel and Arnold [12] and Gulbrandsen.[13] Moreover, the kinds of bacteria isolated from the blood by these investigators were primarily Str. viridans, Str. nonhaemolyticus, the diphtheroids and staphylococci.

Factors responsible for the localization of bacteria from the blood stream around the apexes of the teeth are unknown. In several necropsies many of the teeth cultured either were sterile or failed to show an organism similar to that obtained from the blood stream. On the other hand, in several necropsies only one or two apexes gave positive cultures, while only occasionally did the majority of teeth from a single necropsy give cultures of an organism similar to that isolated from the blood stream. Whether the duration and quantity of bacteria in the blood during life are significant in determining these differences in the apical cultures remains for further study. Recently Weisberger [14] showed that the quantity of Str. viridans inoculated into the blood stream of the rabbit is an important factor in determining the localization of the organism around the incisor teeth.

A chronic inflammatory process at the apexes of the teeth might be a factor determining localization of bacteria from the blood stream. Hill [15] showed in dogs that organisms can be demonstrated after necrosis of the granuloma around the apexes of infected and sterile pulpless teeth. Removal of pulps was shown by Rickert [16] to predispose to localization of organisms in the periapical tissues. Baretta,[17] using dogs and guinea pigs, demonstrated the specific organism in healthy pulp tissue after the visceral and blood cultures had become negative. These observations on the localization of bacteria at the apexes of teeth are not

7. O'Kell, C. C., and Elliott, S.: Lancet **1**:869, 1935.
8. Burket, L. W., and Burn, C. G.: J. Dent. Research, to be published.
9. Fischer, J., and Gottdenker, F.: Wien. klin. Wchnschr. **49**:177, 1936.
10. Kulka, E.: Zentralbl. f. Gynäk. **54**:171, 1930.
11. Desoubry, M. A., and Porcher, M. C.: Compt. rend. Soc. de biol. **47**:101, 1895.
12. Nedzel, A., and Arnold, L.: Proc. Soc. Exper. Biol. & Med. **28**:358, 360, 361, 364 and 366, 1931.
13. Gulbrandsen, L. F.: Am. J. Hyg. **22**:257, 1935.
14. Weisberger, D.: Yale J. Biol. & Med. **9**:417, 1937.
15. Hill, T. J.: J. Am. Dent. A. **19**:1389, 1932.
16. Rickert, U. G.; Chalmers, J. L., and Hadley, F. P.: J. Am. Dent. A. **13**: 1203, 1926.
17. Beretta, A.: Centralbl. f. Bakt. (Abt. 1) **76**:124, 1915.

unexpected, as there are many clinical and experimental observations cited in the literature to show that trauma or an inflammatory reaction in other tissues of the body will readily lead to removal of bacteria and inert particles from the blood stream at these sites.

In some of the necropsies it was difficult to determine whether the bacteremia was a result of dissemination of bacteria from an infected area of a tooth or whether the localization of bacteria in the tissues around the tooth resulted from the infection of the blood stream. Evidence to show that in persons with chronic apical infections of the teeth there may be dissemination of bacteria has been cited.[18] Weisberger [14] showed experimentally that Str. viridans may be liberated into the blood stream from an infected area of an incisor tooth in a sensitized (horse serum) rabbit following a shock dose of horse serum. Further experimental work is necessary, however, to determine whether the physiologic or pathologic changes found within chronically ill persons are also severe enough to cause dissemination from infected teeth.

Other pathways of invasion may play a more important role than the blood stream in determining the presence of bacteria at the apexes of the teeth. Invasion through the dentin, pulp or dental periosteum by the oral bacteria may be the common mode by which Str. viridans, staphylococci and other strains of the flora of the mouth gain entrance to the apexes of the teeth. The slight movements of the teeth during mastication, continual trauma and the constant presence of organisms in the gingival crevices favor the peridental membrane as a pathway of invasion for bacteria from the oral cavity. These findings clearly indicate the need for many more bacteriologic observations to be made on intact, noncarious teeth from necropsies on persons with both negative and positive blood cultures before conclusions may be drawn as to the source of the apical cultures of the teeth.

SUMMARY

Cultures of human cardiac blood, viscera and apical tissues of the teeth obtained at necropsies show no significant differences in the frequency of recovery of bacteria from these sites. The greatest difference occurred in the kinds of organisms obtained.

Str. viridans and Str. nonhaemolyticus were predominant among the bacteria recovered from the tissues around the apexes of the teeth. Other kinds were obtained with greater frequency from the blood stream and viscera. Staphylococci were procured from both teeth and viscera, in about the same frequency.

Only eleven (10 per cent) of the apexes isolated in seven of thirty-eight necropsies on persons with bacteremia had gingival tissue and

18. Reith and Squier.[4] Cecil and others.[5] McEwen and others.[6]

crowns sufficiently well preserved to suggest that the organisms at the apexes were from the blood stream.

B. coli was found around the teeth only in the presence of bacteremia. Str. haemolyticus, D. pneumoniae and three unidentified organisms were the other kinds recovered from both blood and teeth. On the other hand, the relation of bacteremia to the presence of Str. viridans and staphylococci in the periapical areas remains to be determined on further study.

Of the fifty-five necropsies on persons with negative blood cultures obtained clinically or post mortem, eight showed fifteen apexes containing Str. haemolyticus or pneumococci. The infections of three of these apexes are known to have been due to invasion by bacteria from the oral cavity.

Suggestive evidence that transient bacteremia might occur at times during the life of a subject is indicated in the isolation of pathogenic organisms from the viscera post mortem although both the clinical and the postmortem blood cultures remained negative.

Factors influencing the localization of bacteria from the blood stream or their dissemination from infected teeth are briefly discussed.

EFFECT OF CARBON DIOXIDE ON THE CLOTTING OF BLOOD

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Studies reported elsewhere [1] have shown that in experiments on animals the formation and further extension of thrombosis are not favored by periods of inhalation of carbon dioxide. The studies reported here are concerned with the influence of carbon dioxide on the clotting of blood outside the body.

The literature on the influence of carbon dioxide on the clotting of blood is conflicting. Von Recklinghausen [2] counted carbon dioxide as a factor counteracting the clotting of blood. Fano [3] and Wooldridge [4] stated that passage of carbon dioxide through peptone plasma induced clotting. Wright [5] found acceleration of clotting in anesthetized rabbits inhaling carbon dioxide. Pickering and Hewitt [6] found restoration of the coagulability of blood following injections of peptone into pithed cats when they administered short asphyctic periods of from forty to fifty seconds. They ascribed this result to the effect of carbon dioxide. In their physical-chemical theory of the clotting of blood Stuber and Lang [7] considered carbon dioxide the foremost physiologic metabolic factor inducing clotting. In studies on man, guinea pigs and mice, Gerlóczy and Szöke [8] and Marx [9] found acceleration of clotting following breathing of carbon dioxide for short periods and considered their finding a corroboration of Stuber and Lang's theory. On the other hand, there is the old experience of pathologists that the blood

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From the Research Department of the Bender Hygienic Laboratory.

1. Tannenberg, J.: Am. J. Path. **13**:635, 1937.

2. von Recklinghausen, F.: Handbuch der allgemeinen Pathologie des Kreislaufs und der Ernährung, Stuttgart, Ferdinand Enke, 1883, p, 119.

3. Fano, F.: Arch. ital. de biol. **2**:146, 1882.

4. Wooldridge, L. C.: Proc. Roy. Soc., London **38**:69, 1884.

5. Wright, A. E.: Proc. Roy. Soc., London **55**:279, 1894.

6. Pickering, J. W., and Hewitt, J. A.: Proc. Roy. Soc., London, s.B **93**: 367, 1922.

7. Stuber, B., and Lang, K.: Physiologie und Pathologie der Blutgerinnung, Berlin, Urban & Schwarzenberg, 1930; Biochem. Ztschr. **179**:70, 1926; **191**:374, 1927; **212**:16, 1929.

8. von Gerlóczy, G., and Szöke, K.: Ztschr. f. klin. Med. **118**:663, 1931.

9. Marx, J.: Arch. f. klin. Chir. **178**:170, 1934.

in persons dying of suffocation is found liquid at autopsy. Dietrich [10] and Aschoff [11] recently confirmed this fact. Mosco [12] recently reported a delay in the clotting of blood taken from guinea pigs following asphyctic periods of several hours' duration. In studies on the effect of various acids on clotting of blood, Bürker [13] found that carbon dioxide, like other acids, delays clotting.

I was especially induced to study the influence of carbon dioxide on clotting by the following observation: I used to obtain blood for various studies from the carotid artery of the rabbit under the usual precautions and under ether anesthesia. Such blood when properly obtained and kept in paraffin-coated cups or open bottles in the amount of 20 cc. or more remained unclotted, without any anticoagulant, for from twenty to twenty-five minutes at room temperature. In a few instances, when too much ether was given, a rabbit apparently died before the bleeding started. When my assistant and I succeeded, by several minutes of artificial respiration, in reviving the rabbit, so that it began breathing spontaneously again, and then started exsanguination from the carotid artery, the blood flow was unusually slow, and the blood itself was dusky red like that of a vein. But, contrary to our expectation, the clotting of such blood was always markedly delayed. In spite of the influence of ether, which is supposed to accelerate clotting (Geissendörffer [14]), and the apparent saturation of the blood with carbon dioxide, this blood remained liquid for about one hour when kept under the usual conditions.

This experience, though repeated a few times, was not considered a clearcut illustration of the effect of carbon dioxide on the clotting of blood because a multiplicity of other factors seemed to be involved. But it seemed worth while to test the coagulability of venous blood from a large vein of the rabbit by systematic experiment. Thus the blood of the inferior vena cava was drawn from rabbits under similar conditions, i. e., under ether or pentobarbital sodium anesthesia. This blood, kept at the same conditions as carotid blood, remained unclotted on the average for from forty to fifty minutes—about twice as long as blood from the carotid artery. The comparatively slow clotting of the blood from the vena cava was encountered so regularly and found

10. Dietrich, A.: Thrombose, ihre Grundlagen und ihre Bedeutung, in Aschoff, L., and others: Pathologie und Klinik in Einzeldarstellungen, Berlin, Julius Springer, 1932, vol. 4.

11. Aschoff, L.: Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch. **7**:11, 1934.

12. Mosco, M.: Riv. di pat. sper. **13**:319, 1934.

13. Bürker, K.: Centralbl. f. allg. Path. u. path. Anat. (supp.) **63**:185, 1935.

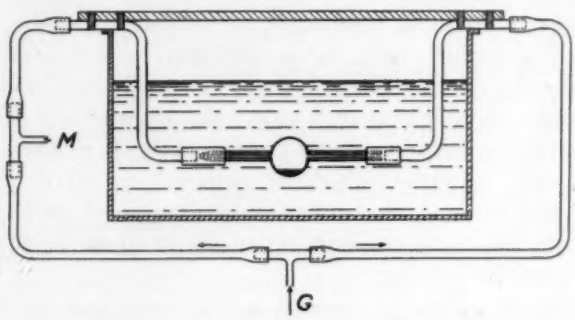
14. Geissendörffer, H.: Deutsche Ztschr. f. Chir. **246**:89, 1935.

so convenient that we adopted this method whenever we needed a larger amount of rabbit's blood for various studies, instead of obtaining it as formerly from the carotid artery.

TECHNIC

Preliminary investigations suggested no method particularly suitable for the study of the influence of gases on the clotting of blood, especially when fresh whole blood, as well as citrated and recalcified blood, was to be used. Therefore, a new apparatus was specifically constructed which eliminated mechanical disturbances of the blood by the administered gas and nevertheless permitted saturation with the gas within a reasonable period of time, compared with the entire duration of the clotting process.

This apparatus, which can easily be constructed in any laboratory with the assistance of a glass blower, is illustrated in the accompanying figure. It consists of a specific tube containing the blood to be studied and a tubing system



A diagram of an apparatus for the study of clotting of blood. It consists, aside from the water bath, of (1) a specific tube containing the blood to be studied and (2) a tubing system connected at *G* with the gas container and at *M* with a mercury manometer.

filled with gas flowing under the same pressure in two opposite directions and arranged to hold the blood tube in a horizontal position.

The specific tube was made of a thick capillary glass tube, the middle portion of which was blown out to a bulb 1 cm. in diameter. Glass capillary tubes having a bore of 1.5 mm. in diameter were employed. The lower ends of these tubes were measured accurately to be of the same length, 37.5 mm. This was to facilitate the comparative measurement of the blood sucked into the bulb. Before use these tubes were usually coated with petrolatum. Thereby sticking of blood to the wall of the capillary tube was prevented, and the clotting rate was delayed, which proved to be advantageous in the study of the influence of gases on clotting. The petrolatum coating helped, also, to prevent air bubbles when the blood was sucked from the capillary into the bulb. For the aspiration of blood into the tube, its upper end was connected with a rubber tube provided with a mouthpiece of glass. For a clotting test the lower capillary end of the tube was entirely filled with blood.

Prior to the connection with the gas tube, a gas current under very low pressure was allowed to flow through the tubing system, already in place above the

water bath. Then, at first, the upper end of the blood tube was connected with the gas tubes so that the gas current could replace the air within the bulb and also remove small remnants of blood within the lower capillary end, if any were present. By the connection of the lower end a few seconds later the system was closed, and the flow of the gas current within the blood tube stopped. The blood tube was then immediately immersed in the water bath. By opening of the valve of the gas container it was possible to raise the gas pressure to any height desired.

A rectangular copper pan containing 10 liters of water was used as a thermostat. It was long and wide enough to allow simultaneous observation of three or four tubes containing blood specimens under the influence of various gases. A turn of the tubes within the water bath about 90 degrees each minute allowed observation of the blood and recognition of the beginning and end of clotting. Beginning of clotting was indicated by the appearance of fine strands of fibrin when the blood was moved during a turn of the apparatus. End of clotting was noted when the whole blood drop was rendered immovable.

RESULTS

Blood from the ear vein and artery, carotid artery, aorta and inferior vena cava of the rabbit and from the heart of the guinea pig was employed. Whole blood was studied directly following removal, also citrated blood and plasma that had been kept in the icebox for various periods of time following removal and recalcified by a 5 per cent solution of calcium chloride just prior to the test.

In specific experiments, it was studied whether the increase in the gas pressure in itself had any effect on clotting. In two tubes containing samples of the same blood, the air was replaced by carbon dioxide. Then both tubes were slowly turned for one minute to permit a large saturation of the blood with carbon dioxide. When the blood had attained the characteristic dusky red-violet color, one tube was shut off while in the other the pressure of carbon dioxide was increased to from 5 to 21 cm. of mercury. Within that range the blood showed no difference in rate of clotting compared with the blood saturated under atmospheric pressure, provided there was no escape of gas from the tubes exposed to the high gas pressure.

In the clotting tests, three tubes with samples of the same blood were usually studied simultaneously, the blood in one being exposed to carbon dioxide, that in the second to oxygen and that in the third to atmospheric air. Oxygen and carbon dioxide were administered under the same pressure, usually from 10 to 12 cm. of mercury above atmospheric pressure. During the clotting period the blood within the air tubes also absorbed some oxygen from the air, as was indicated by the change of color in originally dark venous blood. The effect of the experimentally administered carbon dioxide also varied according to the different carbon dioxide contents of the blood samples obtained from various sources, but always in the direction of retarded clotting. The average delay was from 30 to 50 per cent compared with the rate of clotting in the respective samples exposed to the air. In each sample the evaporation of carbon dioxide and the slowly increasing saturation with oxygen were varying factors depending primarily on the original content of these gases, the temperature and the duration of the clotting test. These factors were not determined in each instance, but other arrangements to be reported aided in their evaluation.

In specific tests it was studied whether or not the saturation of blood with carbon dioxide within physiologic limits had any effect on clotting. Blood taken from the inferior vena cava was placed in a clotting tube, both ends of which

were tightly closed, while another sample of the same blood was left in open communication with the atmospheric air, and a third was placed under pressure of carbon dioxide. The results are reported in table 2.

TABLE 1.—*Effect on Rate of Clotting of Different Test Procedures*

Experiment	Blood Used	Time from Drawing of Blood to Recalcification	Temperature, C.	Rate of Clotting, Minutes		Time from Drawing of Blood to End of Clotting, Minutes	
				Air Tube	CO_2 Tube	Air Tube	CO_2 Tube
3.30	Whole blood from the inferior vena cava, uncitrated	.....	21	20-26	55-65	26	65
	Same blood first exposed 25 minutes to CO_2 then to air	.....	21	42-52	.....	52	..
5.29	Blood from the inferior vena cava, citrated	7 min.	27	48-58	190-208	65	215
	Same blood, after recalcification, first exposed 118 minutes to CO_2, then to air	7 min.	27	43-55 (+ 118)	.....	180	..
	Blood from which 35 to 45 minutes after it was drawn blood gases had been evacuated in vacuo and which had been kept under liquid petrolatum at 27 C.	52 min.	27	14-19	48-68	71	120

TABLE 2.—*Effect on Rate of Clotting of Saturation of Blood with Carbon Dioxide Within Physiologic Limits*

Experiment	Blood Used	Time from Drawing of Blood to Recalcification	Temperature, C.	Rate of Clotting, Minutes		
				Air Tube	CO_2 Tube	O_2 Tube
5.13.-1	Whole blood from the inferior vena cava	.....	23	22-24	24-37	20-24
	Blood citrated and kept under paraffin oil in the icebox	2 days	23	12-15	34-37	12-13
5.18	Whole blood from the inferior vena cava	.....	20	150-181*	300-330†	106-115
	Blood citrated and kept at 23 C. in wide open paraffin-coated cups	10 min.	20	105-118		
5.18	Same citrated blood kept under same conditions	120 min.	20	56-68		
	Blood from which gases had been evacuated for 10 minutes in vacuo	120 min.	20	26-33		
5.18	Citrated blood kept in the icebox	24 hr.	20	15	23	
	Evacuated blood kept in the icebox	24 hr.	20	16	38	

* The air tube was tightly closed at the outer ends of the apparatus so that the original content of carbon dioxide escaped only to a small degree, whereas its escape was facilitated in the oxygen tube and in the citrated blood tested ten minutes later.

† The carbon dioxide pressure was 160 mm. of mercury.

The effect of carbon dioxide was considerably more definite when the blood was citrated following removal and kept in the icebox for one or several days up to five. This caused considerable evaporation of the original carbon dioxide contained in it, and the hemoglobin was slowly changed to oxyhemoglobin.

In further experiments, the original blood gases were removed to a considerable extent by placing the blood, following citration, in a vacuum with a pressure of from 1.5 to 2 cm. of mercury for a period of from five to ten minutes. During that period the blood gases were withdrawn, but not without many gas bubbles being produced within the blood.

Portions of the evacuated blood were subsequently saturated with oxygen or carbon dioxide, and their clotting time was determined following recalcification. For further control, samples of such blood were saturated with other gases, such as illuminating gas and vapor of ether. Saturation with these respective gases was obtained in various ways: 1. The blood was evacuated in separatory funnels which were subsequently filled with the respective gas. 2. After recalcification, it was distributed in tubes of the clotting apparatus, and there saturated in the usual way. 3. Following distribution of from 1 to 2 cc. of the evacuated blood into test tubes 15 cm. high and 1.5 cm. wide, the gas was passed directly through the

TABLE 3.—*Effect on Rate of Clotting of Partial Removal of Blood Gases*

Experiment	Blood Used	Time from Drawing of Blood to Recalcification	Temperature, C.	Rate of Clotting, Minutes				
				Air Tube	CO_2 Tube	O_2 Tube	Ether Tube	Illuminating Gas Tube
5.13-2	Whole blood from the inferior vena cava	.....	21	29-39	39-42	28-37	...	...
	Blood citrated and kept at 23 C.	16 min.	21	26-31	.....	.....	...	...
	Blood from which blood gases had been evacuated in vacuo and which had been saturated with various gases passed through the blood before recalcification	93 min.	21	6-13	8-15	4-6	4-7	...
5.17	Blood from the inferior vena cava, citrated and kept 2 days in the icebox, then subjected to evacuation of its gases and saturated with various gases passed through it before recalcification	2 days	23	.....	16-20	5-7	5-6	5-8

blood for two minutes; then the tubes were tightly closed and kept in the water bath for about ten to fifteen minutes, until all visible gas bubbles had disappeared. Following recalcification, the rate of clotting was determined directly within these tubes, which were removed from the water bath for observation every minute and tilted so that the bottom of the tube could be examined. Strands visible at the bottom indicated the beginning, and immovability of the blood the end, of clotting.

In all these tests carbon dioxide produced marked delay of the clotting as compared with that of blood samples which were exposed to the air or saturated with oxygen, ether or illuminating gas. Table 3 gives an example of such tests.

The rate of clotting of blood evacuated in vacuo cannot be directly compared with that of the same blood prior to evacuation. This is especially true when non-citrated fresh blood is employed, but even in citrated blood the gas bubbles formed during the evacuation exert a definite mechanical effect, especially on the platelets, so that definite acceleration of clotting results. This effect is stronger than the influence of the gases which are removed and subsequently added again in pure form. The example given in table 1 illustrates the mechanical effect clearly.

The systematic determination of the effect of carbon dioxide on clotting of the same blood following the lapse of various periods after its removal from the blood vessel showed that the influence of that gas is not always the same. When the blood was contaminated with a comparatively large amount of tissue juice, the delay of clotting produced by carbon dioxide was very short, so that it might have been overlooked when the clotting process was examined only once a minute. The same was true when a larger disintegration of the platelets took place in the citrated blood at certain periods, such as we found two hours after removal when the blood was kept at a room temperature of from 23 to 27 C. (73.4 to 80.5 F.).

In our experiments on the effect of carbon dioxide on thrombosis [1] the autopsies on rabbits put to death were performed within periods of from ten minutes to two and one-half hours following death, in order to study the influence of the carbon dioxide on the spontaneous clotting within the heart and the large veins. These tests showed that the spontaneous coagulation of blood within the cavities of the heart was slowed down compared with that in rabbits killed by other means. In the large veins the blood was still unclotted two and one-half hours after death. When removed, however, and contaminated with tissue juice, it coagulated well. Here, also, just as in the clotting experiments proper, the saturation of the blood with carbon dioxide produced definite delay of clotting without, however, destroying the ability to clot.

COMMENT

The difficulty of determining the effect of carbon dioxide on clotting of blood was primarily one of method, namely, the prevention of mechanical disturbances by the administered gas. Having constructed the clotting tube with a bulb in the middle as described, I first studied the effect of a gas current flowing under very low pressure through the bulb. Although the gas did not pass through the blood itself, but only moved slowly over its surface for several minutes, this slight mechanical influence was enough to cause definite acceleration of the clotting, regardless of the nature of the gas employed. Obviously this mechanical disturbance had to be eliminated, and the flow of gas over the surface of the blood was confined to a period of a few seconds when the air within the bulb was replaced by the gas. Bürker, studying the rate of clotting in vacuo, found it diminished to three and a half minutes, compared with five minutes for the same blood under atmospheric conditions. He referred this effect to the removal of the normal content of carbon dioxide in the blood. But I am not sure how far the mechanical effect of the gas bubbles formed in the blood in vacuo is responsible for this acceleration.

The experiments reported have shown that carbon dioxide is capable of delaying clotting but cannot prevent clotting when other substances that induce clotting are present in decisive amounts.

The results reported here are apparently directly contradictory to those of other authors, who found acceleration of clotting when they subjected living animals or persons to asphyxia or to breathing of carbon dioxide for short periods. The shortness of the periods during which carbon dioxide was administered in some of these experiments (from

forty to fifty seconds) in itself suggests that another mechanism than saturation of the blood with carbon dioxide is responsible for the acceleration of clotting. This is obviously the effect of carbon dioxide on the peripheral and central nervous system and the endocrine reactions brought about by this irritation. Bolton, Carmichael and Williams [15] showed that short periods of inhalation of carbon dioxide produced an increase of the blood pressure for a short period, followed by secondary dilatation of peripheral arteries. Cordier [16] showed a definite increase of the blood sugar on administration of carbon dioxide. All this is rather suggestive that under administration of carbon dioxide for short periods a larger output of epinephrine or similar principles is produced by means of nervous reflexes. It has recently been shown by various authors (Kanowoka; [17] La Barre and Allard [18]) that weak solutions of epinephrine given intravenously accelerate clotting of blood. Therefore, it is safe to conclude that the acceleration of clotting by administration of short periods of inhalation of carbon dioxide may be considered only an indirect transient effect of carbon dioxide, which disappears when the blood content of carbon dioxide is given sufficient time for an increase, as shown in the experiments reported.

SUMMARY

The influence of carbon dioxide on the clotting of blood was studied in various arrangements. Whole blood obtained from various veins and arteries was studied directly following its removal, in a specific apparatus whereby saturation with carbon dioxide was rapidly secured without mechanical disturbance of the blood by the gas. Citrated and recalcified blood and plasma were studied systematically directly following removal and after various periods of time up to five days. Citrated blood the gases of which were evacuated in vacuo and which was subsequently saturated with carbon dioxide or oxygen was also studied. In all these samples, carbon dioxide produced a varying, but definite delay of clotting without, however, preventing clotting. Accordingly the spontaneous clotting of blood in the heart and large veins of rabbits put to death following periods of inhalation of carbon dioxide was delayed.

Acceleration of clotting following very short periods of inhalation of carbon dioxide is considered an indirect transient effect of carbon

15. Bolton, B.; Carmichael, E. A., and Williams, D. J.: J. Physiol. **88**:113, 1936.

16. Cordier, D.: Ann. de physiol. **10**:1 and 1135, 1935; **11**:162, 1935.

17. Kanowoka, S.: Tohoku J. Exper. Med. **24**:295, 1934.

18. La Barre, J., and Allard, A.: Arch. internat. de pharmacodyn. et de thérap. **51**:93, 1935.

dioxide on the peripheral and central nervous system, producing an enlarged output of epinephrine.

Carbon dioxide is considered one, but not the only or most important, metabolic product which physiologically counteracts clotting and thrombosis, especially in the small veins of the liver and kidney, where practically no thrombosis is encountered in spite of a particularly slow flow of blood, even when progressive thrombosis is found in other veins. Carbon dioxide may also be largely responsible for the fact that blood in the capillaries is found liquid following death. This fact has hitherto been ascribed to the influence of the capillary endothelium, for which theory, however, reasonable evidence was never adduced.

AN EXPERIMENTAL INVESTIGATION OF LECHUGUILLA POISONING

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Serious losses occur among goats and sheep and to a limited extent among cattle in the Southwest as a result of grazing the leaves of lechuguilla (Agave lecheguilla, fig. 1). The constant manifestations of this disease consist of icterus and degenerative changes in the renal and hepatic parenchyma. An edematous swelling of the face and ears similar to that of the photodynamic diseases is frequently, but not constantly, observed. This plant has been known to be poisonous for livestock for a long time, but prior to the present investigation there was no proof that it exercised a photodynamic effect. This article reports the results of an experimental investigation of both the photodynamic and the toxicologic aspects of lechuguilla poisoning as it may be produced in rats, rabbits and guinea-pigs. The natural occurrence of the disease and an experimental study with sheep and goats will be dealt with in articles appearing elsewhere.

I. INVESTIGATION OF PHOTODYNAMIC CHARACTER OF LECHUGUILLA

In reviewing the literature,[1] two clinical types of photodynamic disease of the lower animals can be recognized. In one type, photosensitization is not associated with icterus, and on protection from sunlight mortality is largely prevented. In the other, photosensitization is associated with icterus, and serious losses are of frequent occurrence. In lechuguilla poisoning, icterus is of constant occurrence, making it appear probable that this part of the clinical picture is due to a hepatotoxic substance and is not essential, therefore, to the photosensitization. Such was found to be the case, for, as will appear later in this report, extracts of lechuguilla were found to have a lethal action in the dark as well as in the light, and from the leaves of the plant an additional toxic principle was isolated, having many of the properties of a saponin but no photodynamic action.

From the Department of Pathology, University of Michigan.

1. Mathews, F. P.: Arch. Path. **23**:399, 1932.

EXPERIMENTAL PROCEDURES

Since laboratory animals refuse to eat lechuguilla, it was necessary to introduce the material in the form of extracts of the leaves. A water extract was prepared by soaking the ground green leaves in distilled water for from ten to fourteen hours at room temperature, filtering, and concentrating by boiling. An alcoholic extract was prepared by covering the dried, ground leaves with 96 per cent ethyl alcohol and boiling, with a reflux condensor attached, for from two to three hours. The hot alcohol was decanted off, and the extraction repeated twice. The three alcoholic fractions were combined and filtered while hot and again at room temperature. For use, the alcohol was evaporated and the residue taken up in distilled

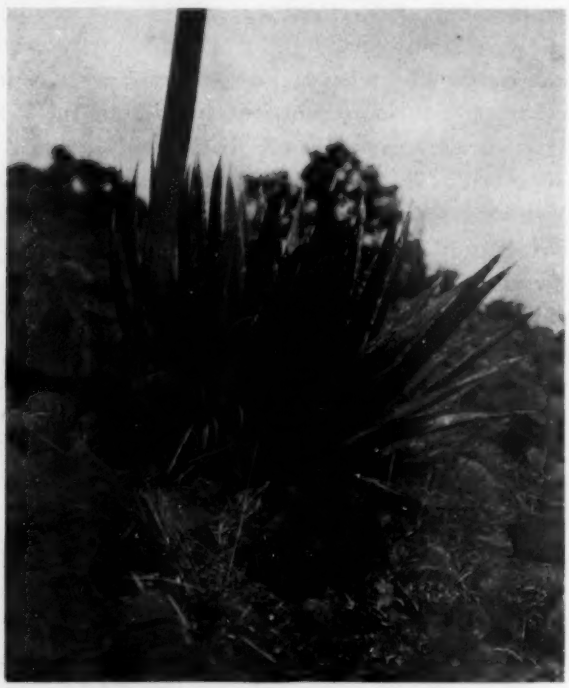

Fig. 1.—Lechuguilla (Agave lecheguilla).

water. The dosage of both the aqueous and the alcoholic extract was computed on the basis of dry solids. For this computation, an aliquot part was spread over a tared receptacle and dried to a constant weight over a steam coil. The extracts were administered to rats and rabbits by stomach tube and to guinea-pigs as boluses, after being mixed with a small amount of flour.

The source of light was direct sunlight except as otherwise specified. Exposures were of two to three hours' duration, depending on weather conditions, and were controlled by simultaneous exposure of nonsensitized animals, care being taken not to produce ill effects in the controls.

Certain comparative experiments were arranged following ingestion of buckwheat (Fagopyrum esculentum) or its derivatives and also following injection of eosin. Rats took an exclusive ration of the ground grain of buckwheat, and

rabbits, an exclusive ration of the whole grain; for guinea-pigs, the ground grain was mixed with ground carrots. Buckwheat hulls were extracted with 96 per cent ethyl alcohol at room temperature and again at boiling temperature. The resulting extracts were combined, filtered and the alcohol evaporated. The dried extract was fed to guinea-pigs as boluses and to rats through a stomach tube, after being mixed with water. Buckwheat middlings were fed to four rats. The experimental use of buckwheat was confined to the ripe fruit or products thereof.

SENSITIZATION WITH SMALL DOSES OF LECHUGUILLA EXTRACT

Rats and guinea-pigs were consistently rendered sensitive to sunlight by the administration of small doses of the extracts of lechuguilla,

TABLE 1.—*Photodynamic Action of Extracts of Lechuguilla*

Animal	Daily Dose, Gm.	Days Fed	Reaction to First Exposure to Sunlight
	Alcoholic Extract		
Rat 51	0.1	34	21st day, positive
Rat 77	0.05	30	5th day, positive
Rat 85	0.025	37	18th day, positive
Rat 88	0.1	8	4th day, positive
Rat 90	0.1	8	3d day, positive
Rat 92	0.1	8	2d day, positive
Rat 89	0.025	8	3d day, positive
Rat 91	0.025	8	2d day, positive
Rat 93	0.025	8	4th day, positive
Rat 97	0.025	6	2d day, positive
Rat 99	0.025	3	24th hour, positive
Rat 104	0.025	1	6th hour, positive
Rat 98	0.012	6	Slight
Guinea pig 15	0.1	15	9th day, positive
Guinea pig 27	0.03	7	3d day, positive
Rabbit 10	2.5	35	No sensitization at any time
Rabbit 11	1.0	20	No sensitization at any time
Rabbit 13	0.25	15	No sensitization at any time
	Water Extract		
Rat 50	0.25	30	18th day, positive
Rat 78	0.25	30	18th day, positive
Rat 46	0.16	30	18th day, positive
Rat 79	0.08	30	18th day, slightly positive
Guinea pig 23	0.1	45	14th day, positive
Guinea pig 24	0.05	45	14th day, positive
Rabbit 8	5.0	30	No sensitization at any time
Rabbit 6	1.0	30	No sensitization at any time

but no sensitization was produced in rabbits. The results are summarized in table 1.

With the alcoholic extract the optimal sensitizing dose lay within wide limits, as no difference in intensity of reaction was detected for doses ranging from 0.025 to 0.1 Gm. The lower level productive of a complete reaction was but little higher than that of the minimal sensitizing dose, as rat 98 was given 0.012 Gm. and exposed to sunlight at the same time as rat 104, which was given 0.025 Gm. Rat 104 responded with the complete clinical picture of the disease whereas rat 98 showed but mild lacrimation with no swelling of the face. The time required for the development of sensitization was relatively short. For instance, rat 104 was given the extract at 7 a. m. and exposed to sunlight at 10 a. m. Profuse lacrimation had developed by 10:30 a. m.,

and swelling of the face was noticeable by 3 p. m. The making of exposures as planned was frequently prevented by unfavorable weather conditions, and as a result many animals had to be maintained on sensitizing doses for several days. Thus it was found that sensitizing doses could be repeated for as many as twenty-one days (rat 51) while the animal was kept in diffuse light, and sensitization would remain unaltered until the animal was exposed to direct sunlight. There was no cumulative effect. The reaction obtained following the administration of several sensitizing doses was no more extensive or severe than that obtained from a single dose.

Similar in all respects to the sensitization obtained with the alcoholic extract was that obtained with the water extract. From the limited trials made, the minimal sensitizing dose lay between 0.08 and 0.16 Gm., an amount at least three times larger than that required to produce the same result with the alcoholic extract. Alcohol was, therefore, the more satisfactory solvent for the extraction of the photodynamic agent.

SIGNS AND SYMPTOMS

Energetic scratching, beginning in some instances as soon as five minutes after exposure to sunlight, was the first evidence of sensitization. The import of this reflex must be controlled by comparison of the test animals with nonsensitized animals, for all rats show a tendency to scratch themselves when exposed to bright light. Repeated scratching in guinea-pigs is of greater significance. Resistant animals (discussed later) and those in which the light available was insufficient to induce a complete reaction presented marked erythema. The erythema was less noticeable when a full reaction, destined to show edema in its later phase, was being produced. In nonresistant animals profuse lacrimation was positive evidence that the exposure was sufficient for completion of the reaction. In resistant animals there was usually sufficient lacrimation to be detected but not enough to cause overflow at the margins of the eyelids. The time required for the appearance of lacrimation varied with the season of the year. In the spring from two to three hours were required, but in the summer from one to two hours were sufficient. Nervous disturbances, manifested by squealing and by frantic charging about the cage, were of more frequent occurrence in guinea-pigs than in rats. While paralysis and death were observed, this result was encountered equally in normal animals which had been subjected to an excessive exposure to light.

In sensitized animals subjected to the first exposure to light profuse lacrimation was followed by edematous swelling of the face. In rats this swelling assumed a hemispheric shape between the eyes, tapering to a point somewhat back of the tip of the nose and from this point extend-

ing downward and backward to a point inferior and posterior to the orbit on each side (fig. 2 *A*, rat at left). Maximal edematous infiltration was never obtained by a single exposure to light, exposures on the second and third consecutive days being required to complete the reaction. The second or third exposure was followed by a similar swelling of the ears, which persisted one or two days longer than that of the face. After the disappearance of the edema, continued exposures to light produced extensive necrosis of the ears but no recurrence of the edema. Exophthalmos developed and ran a course parallel with that of the facial edema. Opacity and ulceration of the cornea occurred in the late spring and summer months but not in the early spring. Edematous swelling of the feet was also observed, but this was of shorter duration than that of the face. The scrotum of the rat showed no reaction other than erythema.

MICROSCOPIC OBSERVATIONS

On microscopic study the initial lesion appeared to affect the endothelium of the cutaneous capillaries. Both the nuclei and the cytoplasm of the endothelial cells became greatly swollen and took a pale stain with hemalum and eosin. This injury to the endothelium was soon followed by edematous infiltration of the surrounding tissue, spreading rapidly through the corium, to the deeper connective tissue (fig. 2 *B*). Various wandering cells, including many plasma cells but practically no eosinophils, accumulated in the edematous areas. The plasma cells rapidly underwent a hyaline change of their cytoplasm. By the third day, when the edema had largely disappeared, regeneration of the capillary endothelium was well under way, some capillaries showing an inner layer of degenerating, necrotic cells and an outer wall of normal-appearing endothelium. Thrombosis of the capillaries was occasionally observed, but this change was not sufficiently extensive to account for all of the necrosis which was encountered. With the necrosis were associated polymorphonuclear leukocytic infiltration, sloughing of the epithelium and scab formation. Microscopically, the necrosis was frequently more extensive than was anticipated on gross examination. The cornea showed extensive necrosis of the epithelium, ulceration and marked polymorphonuclear leukocytic infiltration. Vesicle formation occurred beneath the corneal epithelium and beneath the conjunctiva at the limbus.

DESENSITIZING ACTION OF THE TOXIC SAPONIN OF LECHUGUILLA

Paradoxically, small doses of the extract had a greater photodynamic action than large (toxic or subtoxic) doses. Beginning with doses as large as 0.3 Gm. of the original extract, photodynamic sensitization decreased as the dose approached toxic limits for the rat, namely,

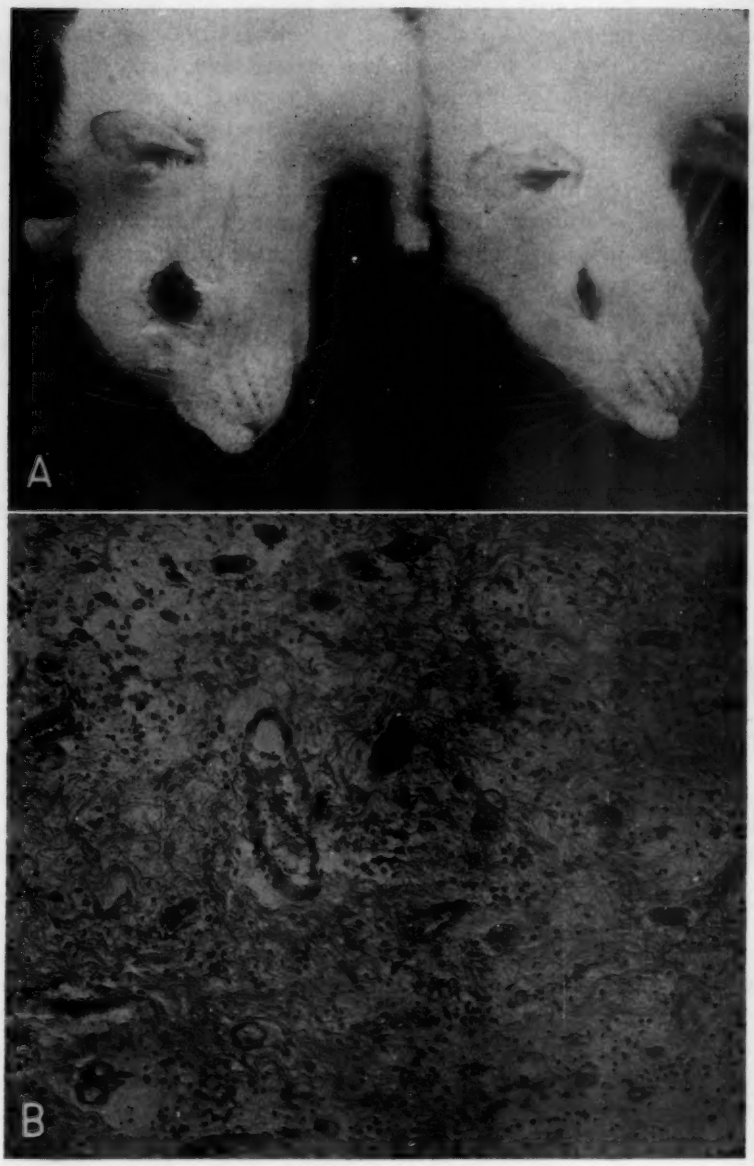

Fig. 2.—In *A* the rat at the left, after being sensitized with extract of lechuguilla, was given three exposures to sunlight. It shows marked edematous swelling of the head with associated exophthalmos. The rat at the right was fed the same extract but not exposed to sunlight. *B* is a section from the head region of a sensitized rat following the third consecutive exposure to sunlight. It shows marked edema of the cutis with swollen endothelial cells and slight cellular infiltration.

0.7 Gm. As described in part II of this paper, the toxic action of large doses of the extract was found to be due to a saponin or a saponin-like substance. This effect could be prevented by hydrolysis and neutralized by adding cholesterol to the extract. After the action of the saponin had been eliminated by either of these methods, photodynamic sensitization could be obtained with as large doses as could be taken. While the reactions obtained by these methods were perhaps a little more pronounced than those obtained with small doses, their intensity was never proportionate to the excess of the photodynamic agent administered, which in the case of a 1 Gm. dose per rat was forty times greater than the minimal sensitizing dose.

TABLE 2.—*Sensitization After Hydrolysis or Neutralization of the Toxic Saponin in the Alcoholic Extract*

Rat	Dose of Crude Extract, Gm.	Dose of Saponin of Crude Extract		Reaction to First Exposure to Sunlight
		Hydrolyzed with Hydrochloric Acid, Gm.	Neutralized with Cholesterol, Gm.	
77	0.05	.....	....	Pronounced
70	0.3	.....	....	Moderate
76	0.4	.....	....	Slight
106	0.4	.....	....	Very slight
71	0.5	.....	....	Very slight
72A	0.5	.....	....	Slight
75	0.57	.....	....	Doubtful
84	0.6	.....	....	Doubtful
87	0.75	.....	....	Doubtful
96	...	0.025	....	Pronounced
104A	...	0.125	....	Pronounced
107	...	0.48	....	Pronounced
105	...	0.5	....	Pronounced
98	...	1.0	....	Pronounced
24	...	.....	0.05	Pronounced
26	...	.....	0.10	Pronounced
27	...	.....	0.5	Pronounced
16	...	.....	1.2	Pronounced
36	...	.....	1.2	Pronounced
30	...	.....	1.2	Pronounced

A portion of the alcoholic extract was hydrolyzed with 0.37 per cent hydrochloric acid and neutralized with sodium hydroxide, and administered in doses as large as 1 Gm. This was equivalent for the rat to 1 minimal lethal dose of the original extract. A saturated alcoholic solution of cholesterol was added to a second portion of the extract at the rate of 0.2 Gm. of cholesterol to each gram of extract. The alcohol was evaporated and the remaining product fed in doses as large as 0.7 Gm. The results are summarized in table 2.

PHOTODYNAMIC ACTION OF BUCKWHEAT

For purposes of comparison with lechuguilla, the photodynamic action of buckwheat (Fagopyrum esculentum) was investigated.

The results of feeding buckwheat (summarized in table 3) confirm those of other investigators who have found that photosensitization

can be produced in this manner (fig. 3 *A*). A prolonged feeding period was not required to produce sensitization, since rats 81 and 83 reacted to exposure to light after having been fed buckwheat for but two days. As with lechuguilla, the sensitization produced by short periods of feeding buckwheat prior to the first exposure to light could not be distinguished from that resulting from longer periods. It was found to be unnecessary to keep the animals in total darkness during the period of sensitization. Rats 9 and 10 were kept in diffuse light, and rats 11 and 12, in a dark room from the beginning of the experiment until the first exposure to sunlight. All four animals exhibited the same degree of reaction. The sensitization produced by buckwheat middlings was similar in all respects to that produced by the whole fruit.

TABLE 3.—*Photodynamic Action of Buckwheat*

Animal	Days Fed	Reaction to First Exposure to Sunlight
Rat 9	85	29th day, positive
Rat 10	85	29th day, positive
Rat 11	105	103d day, positive
Rat 12	85	70th day, positive
Rat 39*	65	25th day, positive
Rat 40*	65	60th day, positive
Rat 41*	65	25th day, positive
Rat 80	14	3d day, positive
Rat 81	14	2d day, positive
Rat 82	14	3d day, positive
Rat 83	14	2d day, positive
Rat 94	4	4th day, positive
Rat 95	4	2d day, positive
Guinea pig 11	56	16th day, positive
Rabbit 4	60	No sensitization at any time
Rabbit 5	60	52d day, slight sensitization
Rabbit 12	7	No sensitization at any time
Rabbit 14	14	No sensitization at any time

* Rats 39, 40 and 41 were fed buckwheat middlings.

The protocol of guinea-pig 11 may be abstracted as providing a characteristic example of buckwheat poisoning.

After a feeding period of sixteen days, this animal was exposed to bright sunlight at 11 a. m. Scratching of the face and ears began at 11:30 a. m. and was continued at irregular intervals until 1 p. m. At that time a nervous disturbance was manifested by squealing and mad charging about the cage. Exposure to light was then discontinued and the animal placed in semidarkness, but the nervous disturbances recurred until about 2 p. m., after which the animal appeared listless for several hours. On the following morning the usual lively attitude was observed. There was now moderate swelling of the face, especially around the eyes; the ears were sensitive to touch but not swollen. Weather conditions prevented an exposure on the second day, and by the third day all evidence of reaction had disappeared. The feeding of buckwheat was continued for several weeks, but the reactions to repeated exposures to light during this period were slight, consisting of erythema, pruritus and lacrimation. The animal apparently had acquired a resistance similar to that observed in many rats.

Four rabbits were fed buckwheat for periods of from seven to sixty days. In but one (rabbit 5) was evidence of sensitization obtained.

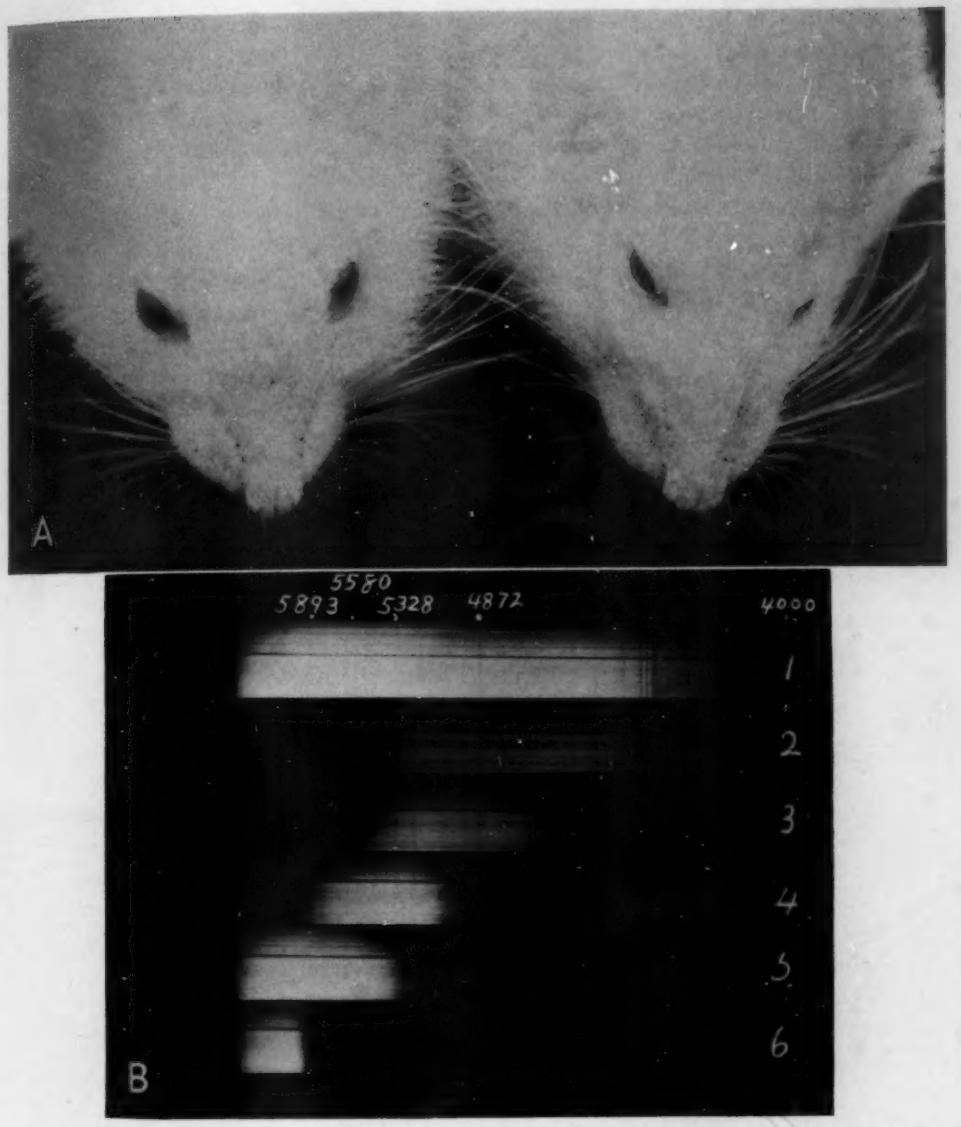

Fig. 3.—In *A* the rat at the left was sensitized with buckwheat and is shown four hours after the second exposure to sunlight. The rat at the right is a normal one that was subjected to the same exposure to sunlight as the rat at the left. *B* presents the transmission of light by the filters employed, compared with the solar spectrum: *1*, solar spectrum; *2*, methylene blue filter; *3*, methyl green B; *4*, Wratten B (green); *5*, acridine yellow; *6*, Wratten A (red). The numbers with several digits indicate wavelengths in angstroms.

The reaction in this animal consisted of erythema and lacrimation, without edema and without evidence of pruritus. Exposure to light was made on the fifty-second day of feeding. Three exposures to light were followed by the same manifestations. After the third exposure the animal was put to death. Microscopic examination showed only congestion of the superficial cutaneous vessels. It will be recalled that no sensitization was obtained in five rabbits with the extracts of lechuguilla. Failure to obtain definite sensitization in rabbits is in keeping with similar results reported by Sheard, Caylor and Schlotthauer.[2]

The photodynamic agent of buckwheat was found to be extractable from the hulls with 96 per cent ethyl alcohol, as is shown in table 4. The results in rats and in guinea-pigs 28 and 29 proved, as with lechuguilla, that the time required for the development of sensitization is relatively short. These animals received the first dose of the extract at 5 p. m. and were exposed to sunlight on the following day at 10 a. m.

TABLE 4.—*Photodynamic Action of Alcoholic Extract of Buckwheat Hulls*

Animal	Amount of Extract Fed, Gm.	Reaction to First Exposure to Sunlight
Rat 69	0.1	18th hour, positive
Rat 70	0.1	18th hour, positive
Rat 73	0.1	18th hour, positive
Guinea pig 26	0.5	6th day, positive
Guinea pig 27	0.5	3d day, positive
Guinea pig 28	0.3	18th hour, positive
Guinea pig 29	0.3	18th hour, positive

The guinea-pigs were scratching themselves at 10:15 a. m., and by 11 a. m. were showing such evidences of distress that the exposure was discontinued. Lacrimation appeared in the rats at 11 a. m.; exposure was discontinued at noon and by 3 p. m. there was noticeable swelling of the face. This extract had no toxic effect other than its photodynamic action, as guinea-pigs 26 and 28 were later fed as much as 5 Gm. at one time and showed no ill effects. Since a single dose of 0.5 Gm. of lechuguilla extract was sufficient to kill a guinea-pig in less than twenty-four hours, it is evident that an additional toxic principle, similar to that in lechuguilla, does not occur to any appreciable extent in buckwheat.

PHOTODYNAMIC ACTION OF EOSIN

Four rats were given intraperitoneal injections of eosin Y and exposed immediately to sunlight. The results of this experiment are summarized in table 5. Two rats which had been sensitized with lechuguilla extract on the preceding day were exposed at the same time.

2. Sheard, C.; Caylor, H. D., and Schlotthauer, C.: J. Exper. Med. **47**:1013, 1928.

With a few minor variations the results from the two methods of sensitization were comparable. Less time was required for the appearance of the reaction in rats 80 and 81 than in the rats sensitized with lechuguilla, but more time than for lechuguilla was required in rats 82 and 83, which had received smaller doses of eosin. Edematous swelling of the face did not appear in rats 82 and 83 until sometime during the night following exposure, whereas in the other animals of the group it was noticeable by 5 p. m. of the same day. At the end of the first twenty-four hours, no difference could be detected in the intensity of reaction in the various animals. Swelling of the ears occurred a little earlier in rats sensitized with eosin than in those sensitized with lechuguilla. As in all previous results, swelling of the face preceded that of the ears, and resorption of the edema occurred in the order of its appearance. Continued exposure to light resulted in the usual necrosis of the ears after the disappearance of the edema.

TABLE 5.—*Photodynamic Action of Eosin After Intraperitoneal Injection*

Animal	Amount of Eosin Injected, Gm.	Result of Immediate Exposure to Sunlight
Rat 80	0.020	Noticeable edematous reaction within four hours
Rat 81	0.005	Noticeable edematous reaction within four hours
Rat 82	0.002	Edematous reaction appeared during the night
Rat 83	0.001	Edematous reaction appeared during the night

RESISTANCE

The exposure of sensitized animals to direct sunlight was followed by the development of resistance in respect to photodynamic effects, regardless of the extent of the reaction produced by the original exposure. The development of resistance was first encountered in a group of twelve rats which were given lechuguilla extract beginning March 1. On March 10, 14 and 17 they received exposure to sunlight totaling six hours, with no visible reaction except pronounced erythema. On March 21, three rats which had been given comparable doses of the same extract but had not been previously exposed to sunlight were exposed with them. On the following day all of the second group showed well developed swelling of the face, but in the first twelve the erythema noted on the preceding day had disappeared leaving no evidence of a reaction. The sensitizing dose was then increased for the group of twelve, but repeated exposures to light failed to produce edema during a period when recently sensitized animals were, without exception, showing a typical reaction.

Resistance was observed also in a group of rats following a single exposure to light. The first exposure resulted in edematous swelling.

A second exposure was delayed for seventy-two hours because of weather conditions. By this time the edema had been completely resorbed, and the lesion could not be reproduced in spite of continued administration of the extract and repeated exposure to sunlight.

Under favorable conditions maximal reactions were obtained by three or four exposures on consecutive days. Under such conditions resorption of edema began by the end of the third day and was generally complete by the end of the fifth day. Recurrence of edema could not be induced by further exposure until sufficient time had elapsed for loss of resistance. The duration of the state of resistance did not exceed ten weeks, and in most cases susceptibility had been reestablished within seven weeks after administration of the photodynamic agent and exposure to light had been discontinued.

The degree of specificity of the acquired resistance was tested as illustrated in table 6. Eight rats resistant to buckwheat were fed lechu-

TABLE 6.—*Specificity of Resistance*

Animals	Sensitizing Agent to Which Animal Was Resistant	Substance Fed	Observation After First Exposure to Sunlight
8 rats	Lechuguilla	Buckwheat	No edema
8 rats	Buckwheat	Lechuguilla	No edema
4 rats	Eosin	Lechuguilla	No edema
2 rats	Lechuguilla	Eosin*	No edema
1 rat	.....	Buckwheat	Pronounced edema
1 rat	.....	Lechuguilla	Pronounced edema

* This was injected.

guilla; eight resistant to lechuguilla were fed buckwheat; four resistant to eosin were fed extract of lechuguilla, and two resistant to lechuguilla were given injections of eosin. On exposure to sunlight, these animals showed the usual erythema and mild lacrimation, but not one of them showed edematous swelling of the head, as did the control animals, which were sensitized with lechuguilla and exposed for the same length of time. Resistance was therefore nonspecific for the agents used.

ACTIVATING LIGHT IN LECHUGUILLA AND BUCKWHEAT POISONING

In determining the region of the activating light, filters were employed consisting of gelatin films in which the desired dye had been incorporated. The films were protected between plates of window glass. The filters used were Wratten A (red), acridine yellow, Wratten B (green), methyl green B, methylene blue and window glass, and later, also methyl orange and trinitrophenol. Two rats were placed beneath each filter, one sensitized with buckwheat and the other with lechuguilla extract. In this experiment, the results of which are summarized in

table 7, exposures to light were continued for thirty minutes after the appearance of profuse lacrimation, previous observations having shown that this was sufficient to produce the edematous reaction. Following the first exposure the groups beneath the acridine yellow filter and beneath window glass responded with the complete reaction, whereas the rats exposed beneath the red, blue and green filters were not affected. On the following days the two groups which had been exposed beneath

TABLE 7.—*Data on Exposure of Sensitized Rats to Filtered Sunlight*

	Filters						
Rat*	Red A	Acridinium Yellow	Green B	Methyl Green B	Methylene Blue	Window Glass	Date Exposed
95	−	..	..	..	..	..	5/6/36
96	−	..	..	..	..	..	5/6/36
81	..	+	..	..	..	..	5/6/36
92	..	+	..	..	..	..	5/6/36
80	..	..	−	..	..	..	5/6/36
89	..	..	−	..	..	..	5/6/36
94	..	..	..	−	..	..	5/6/36
88	..	..	..	−	..	..	5/6/36
82	..	..	..	..	−	..	5/6/36
90	..	..	..	..	−	..	5/6/36
83	..	..	..	..	..	+	5/6/36
91	..	..	..	..	..	+	5/6/36
80	..	+	..	..	..	..	5/7/36
89	..	+	..	..	..	..	5/7/36
94	..	..	−	..	..	..	5/7/36
88	..	..	−	..	..	..	5/7/36
95	..	..	..	−	..	..	5/7/36
96	..	..	..	−	..	..	5/7/36
82	..	..	..	..	..	+	5/7/36
90	..	..	..	..	..	+	5/7/36
94	..	±	..	..	..	..	5/8/36
88	..	±	..	..	..	..	5/8/36
95	..	..	..	..	..	±	5/8/36
93	..	..	..	..	..	±	5/8/36

* The first rat in each group was sensitized with buckwheat; the second, with lechuguilla extract. The sign + signifies edematous swelling of the face; ±, a slight edematous reaction; −, absence of reaction. Red A and green B refer to Wratten filters made by the Eastman Kodak Company.

the green filter B and the methylene blue were exposed beneath the acridine yellow filter and window glass, respectively. The typical reaction developed in both groups. The two remaining groups were exposed on the same day beneath the two green filters, with no reaction. On the third day these two groups were exposed to the full visible spectrum and under the acridine yellow filter, respectively. All four animals reacted, but to a less degree than had the other rats on the preceding days. In repetitions of this experiment, results were consistently negative with red, blue and green light. In addition to the acridine yellow filter, methyl orange and trinitrophenol filters gave positive results.

The milder reaction obtained in the rats exposed on the third day, as described, suggested that partial resistance had resulted from the exposures to red, blue and green light on the preceding days. Accordingly, another group of rats was exposed on consecutive days to red, blue and green light in the order named, and on the fourth day to yellow light. The reaction obtained was of the incomplete form constantly observed in resistant animals, although the quality and intensity of the light were sufficient to induce the full reaction in nonresistant animals. From these results it appeared that of the red, blue and green filters, one or more passed sufficient activating light to produce resistance without having produced recognizable lesions. By increasing the duration of the exposure until nonsensitized controls were showing marked evidence of distress, it was found that lacrimation and later barely perceptible swelling of the face could be produced by both red and green light but not by blue. Therefore the red and green filters did not absorb all of the activating light.

The spectral absorption of the filters listed in table 6 is presented in figure 3 *B*. The activating light is apparently of greater wavelength than 5,328 angstroms, since the acridine yellow filter absorbed practically all light of shorter wavelength. The absorption of the red filter began a little above the sodium lines; that of the green filter B began a little below this region. Therefore, a narrow band was left between these two filters in the region of the sodium lines in which light energy was greatly reduced by the green filter and practically excluded by the red. There was little or no absorption of light in this region by acridine yellow. Since the reactions obtained beneath the acridine yellow filter were equal to those produced by unfiltered sunlight, the activating light for buckwheat and lechuguilla poisoning appears to be located in the region of 5,893 angstroms, provided that the action of a single band of light is all that is required.

ARTIFICIAL LIGHT

Owing to the lack of adequate sunshine during the winter months, attempts were made to demonstrate photosensitization by exposure to light from both carbon and mercury arcs. Methods for sensitization were employed which later proved successful when used with sunlight. Three guinea-pigs, two rabbits and eight rats to which buckwheat had been fed, seven guinea-pigs, three rabbits and fifteen rats to which lechuguilla extract had been fed, and one guinea-pig and seventeen rats which had received intraperitoneal injections of eosin were exposed under carbon and mercury arcs. Except for doubtful pruritus in some of the guinea-pigs, there was no evidence of photosensitization in any of the fifty-six animals exposed to artificial light.

LIGHT ENERGY

Quantitative determinations of the light energy required to produce a complete reaction were not made, but it is evident that this is an important element. Weather conditions permitting, the exposures to sunlight lasted for from two to three hours and were made between 10 a. m. and 2 p. m. With the time of exposure kept as constant as possible, the degree of reaction varied according to the season of the year. The first reaction of edema was obtained in Ann Arbor, Mich., on March 21. The edema which was produced during the latter part of March and through April was insufficient to be detected for at least twelve hours after exposure and in many instances was not noticeable until the morning of the following day. During this period from two to two and one-half hours gave sufficient exposure to complete the reaction. Additional exposure neither increased the extent of the reaction nor hastened its appearance. By the middle of May an exposure of two hours was sufficient to produce a visible swelling of the face by 5 p. m. of the same day, and by 10 p. m. the maximal swelling for a single exposure had occurred. When the investigation was transferred to Alpine, Texas, with use of rats of the same extraction and the same methods of sensitization, reactions produced during July and August were like those obtained in Ann Arbor in May and June. However, by the latter part of September the speed and extent of the reaction had decreased until the reaction was the same as that observed in Michigan in March and April. By the middle of October, exposure for three hours produced marked erythema but no injury to the blood vessels. (This exposure was the maximum that could be tolerated by normal rats on a clear day, even with a cooling breeze.)

The quantitative value of the light energy transmitted by the various filters employed is likewise important in comparing the reactions of sensitized animals under filtered light. This phase of the investigation has not been fully explored. The red Wratten filter A has a total transmission of 15 per cent of the incident light and the green Wratten filter B a total transmission of 26 per cent. The exact transmissibility of the specially prepared filters has not been measured. The percentage transmission of each filter at various wavelengths should be known for a precise evaluation of comparative reactions.

II. INVESTIGATION OF THE NONPHOTODYNAMIC TOXIC PRINCIPLE

TOXICITY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF LECHUGUILLA

Aqueous and alcoholic extracts of Agave lecheguilla were prepared and administered to rats, rabbits and guinea-pigs according to the methods outlined in part I. The results are summarized in tables 8, 9

and 10. It is evident that the toxic principle was soluble in both water and alcohol, but the alcoholic extract proved to be the more toxic for the animals used. Rats were used in sufficient number so that compara-

TABLE 8.—*Toxicity of Water Extracts of Lechuguilla*

Animal	Daily Dose, Gm.	Percentage of Body Weight	Doses	Result
Rat 46.....	0.53	0.21	31	Showed no ill effects and gained weight
Rat 47.....	0.53	0.21	31	Showed no ill effects and gained weight
Rat 2.....	1.06	0.4	7	Ill on 8th day, recovered
Rat 3.....	1.06	0.4	13	Ill on 7th day, died on 14th day
Rat 5.....	1.8	0.53	13	Ill on 4th day, died on 14th day
Rat 6.....	2.0	0.73	2	Ill on 3d day, died on 5th day
Rat 7.....	2.5	0.9	1	Ill on 2d day, recovered
Rabbit 8.....	3.9	0.13	71	Showed no ill effects
Rabbit 6.....	5.3	0.15	82	Lost weight (25%), appeared listless, recovered
Rabbit 3.....	13.5	0.4	13	Very ill on 13th day, recovered
Guinea pig 15	0.2	0.03	50	Showed no ill effects
Guinea pig 18	0.4	0.05	31	Lost appetite on 20th day, died on 44th day
Guinea pig 16	0.53	0.08	22	Lost appetite on 22d day, died on 32d day
Guinea pig 10	0.7	0.12	6	Ill on 2d day, died on 7th day
Guinea pig 3	0.7	0.15	1	Ill on 2d day, recovered
Guinea pig 5	0.8	0.18	9	Ill on 2d day, died on 10th day
Guinea pig 8	1.0	0.23	7	Ill on 2d day, died on 16th day
Guinea pig 2	1.5	0.32	1	Died 28 hours later

TABLE 9.—*Toxicity of Alcoholic (Ninety-Six Per Cent Ethyl) Extract of Lechuguilla*

Animal	Daily Dose, Gm.	Percentage of Body Weight	Doses	Result
Rat 73.....	0.2	0.08	40	Showed no ill effects
Rat 59.....	0.35	0.14	10	Ill on 4th day, died on 11th day
Rat 65.....	0.5	0.2	7	Ill on 4th day, died on 9th day
Rat 56.....	0.5	0.2	6	Ill on 5th day, died on 10th day
Rat 57.....	0.5	0.2	11	Ill on 7th day, died on 14th day
Rat 58.....	0.7	0.28	5	Ill on 2d day, died on 10th day
Rat 60.....	0.8	0.32	5	Ill on 2d day, died on 6th day
Rat 54.....	0.8	0.31	3	Ill on 2d day, died on 7th day
Rat 45.....	1.0	0.43	1	Died in less than 24 hours
Rat 55.....	1.0	0.4	1	Died in less than 24 hours
Rat 61.....	1.0	0.36	1	Died 24 hours later
Rat 64.....	1.0	0.36	1	Died 54 hours later
Rat 66.....	1.0	0.4	1	Died 28 hours later
Rabbit 10....	2.5	0.1	35	Showed no ill effects
Rabbit 9....	5.0	0.28	13	Very ill on 13th day, recovered
Guinea pig 25	0.1	0.01	50	Showed no ill effects
Guinea pig 14	0.3	0.05	1	Ill on 2d day, recovered
Guinea pig 22	0.5	0.08	1	Ill 8 hours later, died in less than 24 hours

TABLE 10.—*Minimal Toxic and Minimal Lethal Doses of the Extracts*

	Minimal Toxic Dose		Minimal Lethal Dose	
Animals	Alcoholic, Gm.	Water, Gm.	Alcoholic, Gm.	Water, Gm.
Rats.....	0.7	1.8	1.0	2.5
Rabbits.....	5.0	15.0		
Guinea pigs.....	0.3	0.7	0.5	1.5

tive results are significant. The minimal toxic dose of the alcoholic extract for rats was 0.7 Gm., and that of the aqueous extract, 1.8 Gm. The minimal lethal dose of the alcohol extract was 1 Gm.; 2.5 Gm. of the water extract produced toxic but not lethal effects. Larger amounts

of the aqueous extract could not be given at a single dose, on account of the volume required. The continued daily feeding of small doses of either extract (alcoholic, 0.2 Gm.; aqueous, 0.53.) was tolerated for long periods without obvious ill effects. However, the continued administration of doses as large as 0.35 Gm. (alcoholic extract) eventually produced toxic effects. With some allowance for individual differences in susceptibility, evidences of toxicity were proportionate to the dosage.

As the tables show, guinea-pigs were found to be much more susceptible than rats. A single dose of 0.5 Gm. of the alcoholic extract was sufficient to kill a guinea-pig in less than twenty-four hours. The greater susceptibility of guinea-pigs as compared with rats was still more evident when the size of the dose was computed as a percentage of the body weight. On this basis the minimal lethal dose for rats was at least five times greater than that for guinea-pigs.

Rabbits appeared to be less susceptible than rats, but definite conclusions could not be reached, on account of the small number of rabbits employed. Five consecutive daily doses, each equivalent to 0.28 per cent of the body weight, were a sufficient amount of the alcoholic extract to kill rat 58, whereas rabbit 9 recovered after 13 doses, each equivalent to 0.28 per cent of its body weight.

EXTRACTION WITH OTHER SOLVENTS

The dry plant was extracted also with methyl alcohol, 70 per cent and absolute ethyl alcohol, acetone, ether and chloroform. The methyl alcoholic and the 70 per cent ethyl alcoholic extracts were prepared as described for the 96 per cent alcoholic extract. For the absolute alcoholic extract the dry plant was first extracted for three days with three changes of anhydrous acetone. After the last acetone had been filtered off, the plant was quickly dried and then extracted with absolute alcohol. The various alcoholic extracts were fed to rats, and the minimal toxic dose was found to be approximately 0.7 Gm. for each, roughly equivalent to the toxic dose of the 96 per cent alcoholic extract. The dry extractives remaining after evaporation of the various fat solvents were insoiuble in water and were therefore fed to guinea-pigs. No ill effects were observed following the repeated feeding of 1 Gm. doses. The toxic principle was not extractable with fat solvents to a demonstrable degree. A summary of these results is presented in table 11.

DETOXICATION OF THE EXTRACTS

With the foregoing information at hand, the toxic principle was assumed to be a saponin. If so, it should be hydrolyzed by dilute hydrochloric acid, precipitated with either barium hydroxide [$Ba(OH)_2$] or

basic lead acetate [$Pb(C_2H_3O_2)_2$] and, according to the suggestion of Autenrieth,[3] cholesterol might prove to render it innocuous. Therefore, an aqueous solution of the alcoholic extract was divided into four parts, the parts treated according to the procedures suggested and the derivatives fed to rats with the results which are summarized in table 12.

One portion was hydrolyzed with 0.37 per cent hydrochloric acid for ten hours, neutralized with sodium hydroxide and the product fed to four rats. (Each rat received daily for from eight to twelve consecutive days the equivalent of 1 minimal lethal dose of the original

TABLE 11.—*Toxicity of Various Extracts*

Extractive	Effect of Extract Fed to Rats	Extractive	Effect of Extract Fed to Guinea Pigs
Alcohol, 70% ethyl	Toxic	Acetone	Nontoxic
Alcohol, absolute	Toxic	Ether	Nontoxic
Alcohol, methyl	Toxic	Chloroform	Nontoxic

TABLE 12.—*Detoxication of the Alcoholic Extract*

Rat	Dose of Extract Hydrolyzed with 0.37% Hydrochloric Acid	Dose of Extract Precipitated with		Dose of Extract Combined with Cholesterol	Result
		[$Ba(OH)_2$]	[$Pb(C_2H_3O_2)_2$]		
69	12 M.L.D.*	.....	.....	.....	No ill effects
70	8 M.L.D.	.....	.....	.....	No ill effects
76	8 M.L.D.	.....	.....	.....	No ill effects
77	8 M.L.D.	.....	.....	.....	No ill effects
72	.....	4 M.L.D.	.....	.....	No ill effects
74	.....	4 M.L.D.	.....	.....	No ill effects
16	.....	.....	4 M.L.D.	5 M.L.D.	No ill effects
36	.....	.....	4 M.L.D.	5 M.L.D.	No ill effects
75	.....	.....	.....	8 M.L.D.	No ill effects
17	.....	.....	.....	6 M.L.D.	No ill effects
30	.....	.....	.....	6 M.L.D.	No ill effects

* This refers to the equivalent of the minimal lethal dose of the original extract, one such equivalent being administered daily to each animal.

extract without ill effects.) To a second portion a saturated solution of barium hydroxide, and to a third, a saturated solution of basic lead acetate, was added. Also 1.5 volumes of 96 per cent alcohol was added to secure a filtrable precipitate. The precipitates were filtered off, and from the filtrates the excess of barium was precipitated with carbon dioxide and the excess of lead with hydrogen sulfide. The filtrates were concentrated by boiling and were found to be nontoxic for rats in 1 Gm. doses. To the fourth portion of the original solution of the extract, a saturated alcoholic solution of cholesterol was added at the rate of 0.2 Gm. of cholesterol to each minimal lethal dose of the extract. The alcohol was evaporated and the residue concentrated until 3 cc. was equivalent

3. Autenrieth, W.: Laboratory Manual for the Detection of Poisons and Powerful Drugs, Philadelphia, P. Blakiston's Son & Co., 1921.

to 1 minimal lethal dose of the original extract. This product was given to five rats, each of which received daily the equivalent of 1 minimal lethal dose for from five to eight days, without ill effects.

The toxic principle (saponin) which had been removed with barium hydroxide was recovered by precipitation of barium with carbon dioxide. After removal of the barium carbonate, 0.7 Gm. of the filtrate produced pronounced toxic effects in a rat which had previously shown no response to the filtrate from precipitation with barium hydroxide. The toxic saponin could not be recovered from the lead precipitate. The filtrate, after precipitation of the lead and removal of the hydrogen sulfide by boiling, was found to be nontoxic. Since the passage of hydrogen sulfide through a solution of the original extract and its removal by boiling did not destroy the toxic principle, it was believed that failure to recover the saponin after precipitation by lead was due to its adsorption by lead sulfide.

PURIFICATION OF THE SAPONIN

An excess of magnesium oxide was added to an aqueous solution of the extract, and the mixture was dried and reextracted with 96 per cent ethyl alcohol. An equal volume of ether was added to the filtered extract and the precipitated saponin redissolved in alcohol and reprecipitated. One gram of the second precipitate was found to be nontoxic for rats, but the filtrate after evaporation of the ether and alcohol was more toxic than the original. To the aqueous solution of the product, a saturated alcoholic solution of cholesterol was added at the rate of 0.5 Gm. of cholesterol to each gram of the extract. The alcohol was evaporated, and the precipitate was filtered, washed several times with water and once with 96 per cent alcohol, and dried for several days in a desiccator. The dried precipitate was dissolved in a small volume of pyridine and 10 volumes of ether added. The white saponin which was precipitated was washed several times with ether and with ether and alcohol in equal parts and then dried for several hours at 90 C. to remove the pyridine as completely as possible. The recovered saponin was suspended in a small amount of water and fed to rats, with results as summarized in table 13. When this table is compared with table 9, it is evident that marked concentration of the toxic principle was obtained in the process of recovering a saponin from its cholesterol compound by this method of Schoenheimer and Dam.[4] The solubility of the purified product in water was much less than that of the original extract. The addition of a small amount of water to the dry powder produced a gel, and further additions of water, an incomplete milky

4. Schoenheimer, R., and Dam, H.: Ztschr. f. physiol. Chem. **215**:59, 1933.

solution. Solubility in water was not increased by heat. Shaking the aqueous solution produced a foam. Erythrocytes were hemolyzed in a dilution of 1:2,500 in physiologic solution of sodium chloride. The purified product was hydrolyzed by hydrochloric acid. Its hydrolyzed products reduced Fehling's solution and were nontoxic. On addition of concentrated sulfuric acid, a red color developed, changing to blue-green. According to some authors, this color reaction is not quite characteristic for saponin. Neither the saponin nor its hydrolytic products were photodynamic.

Other methods of purification of the saponin were tried. Treatment of the alcoholic extract with ether resulted in a precipitate and a filtrate, both of which were toxic. Redissolving and reprecipitating the first precipitate gave a nontoxic precipitate and a toxic filtrate. If either the first or second filtrate was allowed to stand for several days, a second precipitate formed, freely soluble in boiling 70 per cent alcohol, but much of it crystallizing out on cooling. By repeated recrystallization

TABLE 13.—*Toxicity of the Purified Saponin Recovered from the Cholesterol Compound*

Rat	Daily Dose, Gm.	Percentage of Body Weight	Doses	Result
73	0.2	0.06	3	Ill on 3d day, died on 4th day
75	0.2	0.06	3	Ill on 3d day, died on 6th day
80	0.3	0.1	1	Ill on 2d day, recovered

from 70 per cent alcohol a saponin was obtained which had the same properties as that obtained from the cholesterol compound.

A third and still more simple method of purifying the saponin was found. In attempting to obtain a saponin from the methyl alcohol extract by dialysis, according to the method of Rosenthaler,[5] the end-product proved to be nontoxic. It was found that the toxic principle had been precipitated by the chloroform water used in this procedure. This precipitate could be washed with chloroform and recrystallized from 70 per cent alcohol. The end-product was a saponin having the same properties as that obtained by the two preceding methods.

SYMPTOMS

Animals to which the toxic extract had been given assumed a posture indicative of illness and lost weight rapidly even before anorexia developed. Shortly before death breathing was deep and labored but not accelerated. No change in heart action was detected. Death was pre-

5. Rosenthaler, L.: The Chemical Investigation of Plants, London, Bell & Sons, 1930.

ceded by coma. Rats and guinea-pigs to which the original extracts were given presented diarrhea, but this was not due to the toxic saponin, as it did not appear following administration of the purified product.

PATHOLOGIC OBSERVATIONS

In laboratory animals killed by the toxic saponin or put to death while ill, there was no characteristic gross pathologic change. Rats and guinea-pigs dying in from three to seven days after receiving the extract showed mild congestion of the gastro-intestinal mucosa; later there was only increased production of mucin. The only constant lesion was cloudy swelling of the liver and kidneys, with varying congestion of these organs.

Microscopically, the gastro-intestinal mucosa showed cloudy swelling with slight focal necrosis and desquamation. The submucosal vessels were congested, and the villi were rendered plump by the increase in blood and by interstitial edema. Passive congestion and parenchymatous degeneration of the liver and kidneys occurred in all except the animals with very acute poisoning. In rats, the hepatic and renal parenchyma showed albuminous degeneration and moderate focal necrosis, while lipid changes were rarely observed. In guinea-pigs and rabbits, the parenchymatous cells of the liver and kidneys showed marked degenerative fatty infiltration and focal necroses—lesions which occur constantly in sheep and goats as a result of grazing lechuguilla. Renal casts were of regular occurrence, in many cases obstructing the tubules and producing local tubular dilatation. There was no evidence of erythrocytolysis, red blood cell counts remaining normal. A moderate polymorphonuclear leukocytosis was present in some animals.

III. COMMENT

Lechuguilla poisoning is due, therefore, to the combined action of two principles, one a photodynamic agent and the other a nephrotoxic and hepatotoxic saponin. The serious losses of poisoned livestock which frequently occur are due primarily to the degenerative lesion of the kidneys and not to the photosensitization. It is not unreasonable to assume that the losses from some of the other photodynamic diseases which are associated with icterus are due to the same, or a similar, toxic principle. Bile has been shown to have a photodynamic action under certain conditions, but with lechuguilla poisoning there is no reason for attributing the photosensitization to the action of bile pigments.

Chemical identification of the photodynamic agent was not attempted. The extracts after removal of the saponin had a brownish

red color and exhibited a greenish yellow fluorescence in ultraviolet rays. That the activating light for both buckwheat and lechuguilla poisoning is located in the same spectral region is suggestive evidence that the causative agent is the same. Our localization of the activating light for buckwheat poisoning accords with the results of Sheard and his collaborators,[2] who concluded that the specific light was located between 5,800 angstroms and the red end of the spectrum. Rimington and Quin [6] concluded that the photodynamic agent in "geeldikkop" is phyllo-erythrin. They listed four spectral bands for this substance (6,352, 5,950-5,898, 5,580, 5,266-5,123 angstroms) and gave special attention to the region of 5,580 angstroms. From comparison of the absorption bands of phyllo-erythrin with the location of the activating light in lechuguilla and buckwheat poisonings, the possibility that phyllo-erythrin is the photodynamic agent in these diseases cannot be excluded. The region of spectral absorption for phyllo-erythrin between 5,950 and 5,898 angstroms appears to include the most essential light if the two diseases are produced by a single band of light and if phyllo-erythrin is the photodynamic agent. Since acridine yellow permitted the passage of light at about 5,328 angstroms, the light passed by this filter included the three spectral absorption bands of phyllo-erythrin of 5,580 angstroms or greater. That reactions equal to those obtained with unfiltered light were obtained by filtration with acridine yellow may be due to the action of two or more specific absorption bands, whereas the mild reaction obtained by overexposure beneath the red and green filters may be due to the action of a single band of specific light.

Failure to produce photosensitization with artificial light is not surprising, since reactions could not be produced by direct sunlight after October 15 and before March 21. The reproduction of the disease under experimental conditions and the development of resistance were fully in accord with the occurrence of the photosensitization in lechuguilla poisoning under conditions on the range.

SUMMARY

The oral administration of alcoholic and aqueous extracts of Agave lecheguilla rendered guinea-pigs and rats, but not rabbits, sensitive to exposure to sunlight. Small doses of the alcoholic extract proved to be more productive of sensitization than large doses. Less than six hours was required for the development of sensitization.

The feeding of the ripe fruit of buckwheat (Fagopyrum esculentum) and also the alcoholic extract of the hulls of buckwheat rendered rats and guinea-pigs sensitive to sunlight. Buckwheat middlings also had

6. Rimington, C., and Quin, J. I.: Onderstepoort J. **3**:137, 1934.

a photodynamic action on rats. Satisfactory sensitization was not obtained in rabbits.

The intraperitoneal injection of 0.001 Gm. of eosin was sufficient to sensitize a rat to sunlight.

The signs and symptoms were the same regardless of the photodynamic agent employed. They consisted of erythema (as evidence of resistance, or of insufficient light energy to complete the reaction); pruritus; lacrimation; edematous swelling of the face and ears with exophthalmos, followed by superficial necrosis of the ears and opacity and ulceration of the cornea, and nervous disturbances.

The primary change appeared to be hydropic degeneration of the cutaneous capillary endothelium, followed by edema of the neighboring tissues, spreading through the corium and deeper connective tissue. Leukocytes and plasma cells were abundant in the edematous area.

The greater part of the activating light for buckwheat and lechuguilla poisoning was absorbed by red, blue and green filters, but not by yellow. The activating light appeared to be located in the region of the sodium lines. Reactions could not be produced in sensitized animals by exposure to either carbon or mercury arcs.

Recovery was followed in rats and guinea-pigs by a state of resistance in which only erythema could be induced. Resistance could be maintained indefinitely by continued administration of the photodynamic agent and exposure to light. Normal susceptibility was reestablished within ten weeks after discontinuance of these methods. Resistance was nonspecific.

In addition to the photodynamic agent, another toxic principle was extractable from lechuguilla with water, ethyl alcohol and methyl alcohol. This principle was hydrolyzed with hydrochloric acid and precipitated by barium hydroxide and by lead acetate, and it formed a nontoxic compound with cholesterol. It was recoverable in purified form from its cholesterol compound by solution in pyridine and precipitation with ether. Its hydrolyzed products reduced Fehling's solution. Its aqueous solutions produced a foam, and it hemolyzed erythrocytes. It was therefore considered to be a saponin or a combination of saponins.

The minimal toxic dose and the minimal lethal dose of each extract of the plant and of the purified saponin were determined for rats and guinea-pigs, and the saponin was shown to be hepatotoxic and nephrotoxic.

The complete picture of lechuguilla poisoning is, therefore, due to the combined action of two toxic principles: one a photodynamic agent, the other a saponin which has a damaging effect on the liver and kidneys. The serious losses under conditions on the range are due primarily to the saponin and not to the photosensitization.

LYMPHOCYTIC RESPONSE OF GUINEA-PIGS TO EPINEPHRINE HYDROCHLORIDE, SODIUM BICARBONATE SOLUTION AND A CULTURE OF BACILLUS COLI

WITH PARTICULAR REFERENCE TO THE EFFECT OF ANTINEUTROPHILIC SERUM ON THE LYMPHOCYTIC CURVES

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Following the development of an antiserum against the neutrophils of the guinea-pig,[1] the question arose as to what effect the removal of these cells from the peripheral blood would have on the response of the lymphocytes to different substances known to have certain definite effects on the white blood cell picture. The present communication deals with the results obtained with the use of epinephrine hydrochloride subcutaneously, sodium bicarbonate solution intraperitoneally and a broth culture of Bacillus coli intraperitoneally.

LYMPHOCYTIC VALUES OBTAINED IN NORMAL CONTROL ANIMALS

Throughout this investigation, antineutrophilic serum was given intraperitoneally at approximately 4 p. m. On the following morning, a count of the total number of white blood cells and a differential count were made at or about 9 a. m. All injections of the different substances affecting the white blood cell picture were made at 10 a. m., and studies of the white blood cells were made always at one or more of the following intervals after injections—two, three and six hours. Further observations were made at twelve hours in a few instances. Inasmuch as the antiserum was known to have its maximal effect during the six hour period beginning at 10 a. m., standard counts were obtained for

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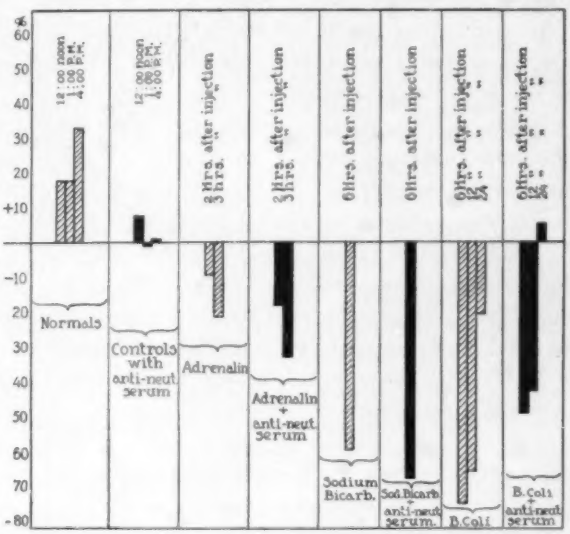
1. Chew, W. B.; Stephens, D. J., and Lawrence, J. S.: J. Immunol. **30**:301, 1936.

this period by the use of twelve normal guinea-pigs. These animals were selected after sufficient preliminary daily studies of their white blood cells had been made to establish that the values were normal. Then during a control day total white blood cell and differential counts were made at 9 a. m., 12 m., 1 p. m. and 4 p. m. From these results, the average percental variations from the values obtained at 9 a. m. were calculated. These values are shown in the first column of the accompanying chart.

As a further control, five "standardized" guinea-pigs were given 0.5 cc. of antineutrophilic serum at 4 p. m., and total and differential white cell counts were made on them on the following day at 9 a. m.,



Lymphocytic average percental variations from the 9 a. m. values at various intervals after injection of epinephrine hydrochloride, sodium bicarbonate solution and a broth culture of B. coli. The solid black blocks represent the figures obtained when the antiserum was administered, whereas the diagonally lined blocks represent the values obtained in normal animals without antineutrophilic serum.

12 m., 1 p. m., 2 p. m., 3 p. m. and 4 p. m. The average percental variations of the lymphocytic counts as compared with the 9 a. m. values are shown in the second column of the chart. It is seen that there was close approximation to the values obtained at 9 a. m. Only the values obtained at 12 m., 1 p. m. and 4 p. m. are shown, as these were the values with which comparisons were made after injection of the different substances used as stimuli.

VALUES AFTER SUBCUTANEOUS INJECTION OF EPINEPHRINE HYDROCHLORIDE

Twelve guinea-pigs were given epinephrine hydrochloride (1 : 1,000) subcutaneously at 10 a. m., nine being given 0.2 cc. and three 0.1 cc. In preliminary experiments it was found that the time of maximal effect on the white blood cell count was two and three hours after injection. Accordingly, these animals, which had previously had daily studies of the white blood cells, had blood counts at 9 a. m. and then at the second and third hours after injection or at 12 m. and 1 p. m. The average percental variations of the lymphocytic values from the figures obtained at 9 a. m. were determined and are shown in the third column of the chart. It is seen that these twelve animals showed a slight average drop in lymphocytes two and three hours after injection. While there were both plus and minus variations in the individual animals, it is felt that the average probably represents the true trend of the number of lymphocytes under these conditions.

Then, fifteen "standardized" guinea-pigs were given epinephrine hydrochloride subcutaneously at 10 a. m. on the day following the injection of antineutrophilic serum. At this time, the animals had no or only few neutrophils in their peripheral blood. Eleven of these animals received 0.2 cc. of epinephrine hydrochloride (1 : 1,000) and four 0.1 cc. Blood counts were made at noon and 1 p. m. These values were compared with those obtained at 9 a. m. of that day, and the average percental variations of the lymphocytic counts were obtained. It is seen (chart) that these values closely approximate those for normal animals given epinephrine.

VALUES AFTER INTRAPERITONEAL INJECTION OF SODIUM BICARBONATE SOLUTION

A previous report from this laboratory [2] has shown that six hours after the intraperitoneal injection of this material there is an average drop of 59 per cent in the lymphocytes.

Six "standardized" guinea-pigs were given sodium bicarbonate solution on the day following the injection of 0.5 cc. of antineutrophilic serum. Three of these animals were given the material in two instalments (10 a. m. and 12 m.), and the remaining three received 10 cc. of a 7.5 per cent solution at 10 a. m. Total and differential counts were made at 4 p. m., or six hours after the injection, and the average percental variation from the 9 a. m. values was obtained. This was found to be minus 68 per cent. Other than a drop in the eosinophils, no changes of note were found in the other white blood cells.

2. Lawrence, J. S., and Josey, A. I.: Folia haemat. **48**:330, 1932.

VALUES AFTER INTRAPERITONEAL INJECTION OF A BROTH CULTURE OF B. COLI

Five standardized guinea-pigs were given 1 cc. of an eighteen hour broth culture of B. coli at 10 a. m., and studies of the blood were made six, twelve and twenty-four hours later. The average percental variations of the lymphocytic values from the 9 a. m. values were calculated and are shown in the chart.

An exactly parallel experiment was run on six guinea-pigs on the day following the injection of 0.5 cc. of antineutrophilic serum. The average percental variations from the 9 a. m. values are shown in the chart.

COMMENT

Reference to the chart shows that the variations in the number of the lymphocytes following the subcutaneous injection of epinephrine hydrochloride and the intraperitoneal injection of a 7.5 per cent solution of sodium bicarbonate follow almost the same course in animals under the influence of antineutrophilic serum as in normal animals. While there is not as close an approximation when colon bacilli are used, yet the general trend is identical and, if the results obtained in one animal of the group that reacted differently from the others are excluded, the percental variations will come within the limits of error of those found in normal guinea-pigs given 1 cc. of a broth culture of B. coli. Thus, it is seen that the absence of the neutrophils from the peripheral blood has in no way affected the response of the lymphocytes to these three types of stimuli. In the case of epinephrine, normal animals responded at from two to three hours with neutrophilic leukocytosis and only slight diminution in the number of the lymphocytes. With sodium bicarbonate solution there was neutrophilic leukocytosis with an average percentage drop of 59 in the number of the lymphocytes. On the other hand, the broth culture of B. coli produced at six hours lymphopenia without neutrophilic leukocytosis, but at twelve hours neutrophilic leukocytosis with lymphopenia and at twenty-four hours neutrophilic leukocytosis with a lymphocytic level close to normal.

The fact that the response of the lymphocyte under all of the aforenamed conditions was approximately the same in the animals treated with antiserum as in the normal animals points strongly to the reaction of this cell as being independent of that of the neutrophil *in the conditions studied*. We feel that our results indicate that the same is true for the other white blood cell constituents but, since the absolute number of these cells is so small in most instances, we do not consider that the series is large enough to make any positive statement in this connection.

We recognize the fact that the absence of the neutrophils from the peripheral blood does not preclude the fact that they may be produced

in normal amounts only to be destroyed as soon as manufactured. All that can be stated from our results is that the absence of these cells from the peripheral blood has no influence on the curve for the lymphocytes resulting from the use of the stimuli reported in this paper.

It would be interesting to extend this study to conditions in which there has been a prolonged stimulating effect on the bone marrow such as Wiseman, Doan and Erf [3] reported for sodium nucleinate. The nearest approach to stimulation of the bone marrow which is noted in this report is at the twenty-four hour interval after the injection of B. coli. One naturally considers that at this time there would have been true stimulation of the bone marrow, yet the response was essentially the same as with epinephrine and sodium bicarbonate, chemicals both of which may be considered as having caused their response through redistribution, although this is by no means certain.

SUMMARY

The lymphocytic response of guinea-pigs given antineutrophilic serum in sufficient quantities to remove the neutrophils from the blood stream is the same as that obtained in normal animals when epinephrine hydrochloride, sodium bicarbonate solution or a broth culture of B. coli is given. In other words, the number of lymphocytes in the peripheral blood is independent of the number of neutrophils in the peripheral blood *under the conditions of these experiments.*

3. Wiseman, B. K.; Doan, C. A., and Erf, L. A.: J. A. M. A. **106**:609, 1936.

Case Reports

TERATOMA OF THE THYROID GLAND

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Teratoma of the thyroid gland, although rare, is among the tumors found most frequently in newly born infants. Pusch and Nelson [1] in 1935 examined critically the cases reported up to that time and accepted as authentic cases of teratoma twenty-eight of the forty-three described. Several of the other cases, refused because of incomplete microscopic examination of the tissues, probably also belong in this group.

The branchiogenic region (particularly that involving the thyroid) and the sacrococcygeal region are the sites of predilection for teratoma in the newborn. In the adult, teratoma arises most frequently in the gonads. In both age groups, however, teratoma may occur in almost any location, practically the only exceptions being the liver, heart and limbs.

An entirely satisfactory explanation of the origin of teratoma has never been proposed. The older theories postulate (1) the isolation of a blastomere in the early stages of cell cleavage, which retains its totipotentiality and migrates to a point where it later develops; (2) the fertilization of a polar body, which subsequently invades the developing embryo; (3) monozygotic twinning, with overgrowth of one twin. Other theories include the belief that multipotent cells from the primitive germ layers are displaced and are enmeshed within tissues undergoing fusion or invagination; that primitive sex cells either in normal or abnormal locations undergo parthogenesis; that soma cells accompanying the germinal cells in their migrations are multipotent and are the origin of tumors. Ewing [2] commented on the fact that belief in the inviolable specificity of germ layers is waning, and the fact that this specificity may not be inviolable is in itself possibly sufficient to explain the mode of formation. Krafka [3] based his conclusions on Spemann's "organizer theory" of embryology and expressed the belief that the teratoma is caused by interference with the normal effect of the "organizer" in the embryo with resultant formation of a secondary embryonic axis in the primitive shield and subsequent development of this into teratoma.

It is possible that a different type of origin may be ascribed to the teratoma in different locations; for example, if the tumor arises in a

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1. Pusch, L. C., and Nelson, C. M.: Am. J. Cancer **23**:791, 1935.

2. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1931, p. 1009.

3. Krafka, J.: Arch. Path. **31**:756, 1936.

gonad, it may have its origin in an abnormal development of sex cells, or if it occurs in the branchiogenic or coccygeal regions, it may have its origin in an inclusion of pluripotent cells within the tissues of these regions during the complex changes occurring in them early in embryonic life.

A theory not previously proposed, which might apply to the limited group of tumors of the thyroid, is that their origin is dependent on abnormal development of the ultimobranchial bodies. These bodies, which are derived from the fifth branchial pouch and which in man normally degenerate during fetal life, lie for a short time close beside the thyroid. In many mammals and occasionally in man a cyst which is believed derived from this body (although some authors believe its origin is from the fourth or the fifth pharyngeal pouch) lies within the thyroid, is usually lined by ciliated epithelium and contains mucous glands in its wall.[4] It occurs in the very young fetus and is rarely seen after the fetus measures 55 mm. from crown to rump. It is possible that this structure under some circumstances might persist and produce a wider variety of tissues, resulting in teratoma.

The fact, however, that teratoma is not sharply separable from a much larger group of abnormalities leads one to believe that not only are all tumors of this type produced by a fundamentally similar process, but that the entire group may well have some etiologic factors in common. The occurrence of all transitional stages between fused symmetrical twins, fused asymmetrical twins, fetal inclusions, reduplication of individual portions of the body (head, jaw, fingers) and tumors containing recognizable fetal structures, on one hand, and tumors composed of recognizable tissue from three germ layers but without evidence of organogenesis, on the other, indicates that all of these conditions have much in common. Since teratoma cannot be sharply isolated into a group by itself, it must, probably, be explained in a way similar to that of the remainder of the group of abnormalities, whatever that explanation eventually proves to be.

Inasmuch as neoplasms are of such rare occurrence in newly born infants, it was deemed advisable to add this case of teratoma of the thyroid to the list of recorded instances. It does not present any features not previously described, but it is an excellent example of a typical case.

REPORT OF A CASE

C., a girl, was delivered at the Chicago Lying-in Hospital Nov. 23, 1935. The mother was white, aged 34, a primipara pregnant for the second time; her last menstrual period began 243 days before the date of delivery. On November 22 she had a small vaginal hemorrhage, and examination revealed a partial placenta praevia. The membranes were ruptured, and a Vorhees bag was inserted. Following expulsion of the bag, dilatation of the cervix was completed manually and the infant delivered by means of a version followed by extraction. Fetal heart tones had been absent about one hour before the infant was delivered. At the time of delivery a large firm tumor mass was present in the right side of the neck.

4. Grosser, O., in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, vol. 2, p. 471.

Necropsy.—The body was that of a white infant, a girl, weighing 2,010 Gm. and measuring 45 cm. from crown to heel and 30.5 cm. from crown to rump. The external surface of the body appeared normal except for moderate generalized cyanosis and a swelling in the right submaxillary region (fig.) This swelling proved to be a tumor mass extending from the clavicle to the mandible, freely movable and apparently attached to fixed structures only medially, in the region of the trachea. It was slightly irregular in form and felt relatively solid to the touch except where a few small cysts were present.

The photograph shows the general location of the teratoma of the thyroid.

On reflecting the skin from the anterior surface of the neck, the left lobe of the thyroid was found in its usual location. The isthmus extended across the trachea, but the right lobe was not visible, and the thyroid tissue of the isthmus became lost on the surface of the mass. The right sternomastoid muscle was thinned out and stretched over the anterior surface of the tumor. The trachea bounded it medially, the jugular vein laterally and the carotid artery posteriorly. The tumor was easily removed by blunt dissection and had no direct connection with any structure except the thyroid. It had numerous very small blood and lymph vessels extending to it from the surrounding connective tissue but none of any considerable size. After removal the tumor measured approximately 7 by 4 by 4 cm. On section irregular masses of solid tissue with intervening cystic areas

were visible. The cysts showed various contents, some containing sebaceous material, some a thick mucin-like substance and others a thin, watery fluid. Most of the solid areas of the tumor were soft and friable, although small cartilaginous islands were also present.

The remainder of the autopsy disclosed that death apparently occurred from anoxemia due to premature partial separation of the placenta, produced as a result of its location.

Microscopic Examination.—The histologic structure of the tumor varied somewhat from section to section, each of ten blocks examined having some specific character not present in any other block. In general, however, the tissue background was surprisingly uniform in all blocks. The great bulk was finely fibrillar in structure and contained numerous cells and nuclei that were characteristic of glial tissue. The fibers with a phosphotungstic hematoxylin stain took on an appearance characteristic of this tissue. Dividing the nerve tissue into individual lobules were connective tissue cells, smooth and striated muscle and a few blood vessels. On the surface of the tumor a thin layer of flattened thyroid acini was discernible. The cystic areas scattered through the tumor varied markedly in their structure. They were lined variously by simple cuboidal epithelium composed of cells that were flattened and thin, without cell boundaries, or large and square, with definite cell margins, and by columnar epithelium that sometimes showed definite evidence of secretory activity and at other times was composed of compressed cells almost entirely filled by nuclei and showing little evidence of secretion. There were cysts lined by pseudostratified cuboidal and columnar cells and others lined by definite stratified squamous epithelium. Most of the squamous epithelium was embryonic rather than adult in type and was similar to that found in the skin of fetuses from 2 to 3 cm. in length. In some areas small bulbous projections were present like those which are characteristic of the first stages in the development of hair follicles.

Some of the cysts which were lined by a single layer of cells had a smooth regular surface; others showed papillary ingrowths. The ingrowths were either small delicate structures, bearing a great resemblance to choroid plexus, or larger, more irregular ones having a connective tissue stroma with considerable similarity to the fimbria of the immature fallopian tube. In some of the cysts with a smooth inner margin the cells were large, secreted mucus and showed numerous regular downgrowths characteristic of the early glandular formation of the intestinal tract in the very young fetus.

There were a few cavities each of which was surrounded by a wide layer of densely packed cells, having large hyperchromatic nuclei and indistinguishable cell boundaries. In this layer of cells were elliptical openings, set at regular intervals, with the longitudinal axes at right angles to the lumen of the larger cavity. About the smaller, secondary cavities the cells were somewhat more densely packed than in the intervening areas.

In addition to the large amounts of glial and connective tissue and smooth and striated muscle, there were numerous other structures in the solid areas. There were masses of cartilage of various sizes and shapes. In one region these were very small and were associated with a few mucus-secreting glands and narrow irregular cavities lined by tall columnar epithelium. This association makes one wonder whether this represents an abortive attempt at formation of a lung. In another area were cross sections of a group of tubular structures in close association with a mass of cuboidal cells, interspersed and divided by cells resembling endothelium. The appearance suggested renal tubules and abnormal glomeruli.

In many portions of the tumor there were noncystic masses of cells having no resemblance to any organ. They probably represented various types of relatively undifferentiated embryonic tissues.

Scattered throughout the tumor, particularly in the last mentioned areas, were numerous masses of calcium. These appeared entirely extracellular and had no relation to any recognizable fetal structures.

The types of tissue found in the tumor may be summarized as follows:

A. General types:
   1. Undifferentiated embryonic cells
   2. Glial cells and fibers
   3. Smooth muscle
   4. Striated muscle
   5. Connective tissue
   6. Cartilage
   7. Mucous glands

B. Cysts containing:
   1. Cuboidal epithelium, (*a*) flat, (*b*) high, (*c*) pseudostratified
   2. Columnar epithelium
   3. Squamous epithelium with immature hair follicles
   4. Papillary and adenomatous structures

C. Attempts at organ formation:
   1. Kidney
   2. Gastro-intestinal tract
   3. Lung
   4. Brain

General Review

ROLE OF VITAMIN B IN RESISTANCE

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(Concluded from page 568)

VIII. EFFECT OF DIETS RICH IN VITAMIN B ON THE NATURAL RESISTANCE OF ANIMALS TO EXPERIMENTAL INFECTION

The addition of preparations and foods rich in vitamin B_1 to the diets of mice greatly increased their resistance to the virus of yellow fever introduced intracerebrally. Kuczynski,[67a] in an extended series of carefully planned experiments on about 5,000 mice, studied the effect on this infection of variations in apparently adequate diets.

The general method used was as follows: The virus was introduced into the brains of the mice. The brain tissue was emulsified in saline solution, thoroughly shaken, and then centrifuged for a specified time and at a constant rate. The supernatant fluid was diluted 1:10 and used for inoculations within from three to four hours. The amount of emulsion injected was constant throughout the experiments (from 0.01 to 0.02 cc.). The virus was repeatedly tested on monkeys and was used in these experiments only after several passages through mice. Mice were tested in groups of from 20 to 25. Unfortunately the mice were not all drawn from the same strain, but care was employed to avoid epidemics of infections with Bacterium aertrycke and Corynebacterium pseudotuberculosis murium.

The diets employed were varied. The standard diet consisted of oats and milk, butter fat and the McCollum salt mixture. The animals were maintained on the diets, beginning from the time of weaning, for from seven to eight months before being inoculated with the virus.

Of 671 mice fed on the normal or standard diet, 20 per cent survived the inoculation of the stated quantity of virus emulsion. The incidence of survival in mice maintained on some of the diets rich in wheat derivatives was as high as 75 per cent. On the other hand, in mice fed diets relatively poor in vitamin B_1, such as one consisting of casein and rice and partially freed from vitamin B_1, the incidence of survival was only

From the Laboratory Division of Montefiore Hospital.

67a. Kuczynski, M.: The Alimentary Factor in Disease, The Hague, G. Naeff, 1937.

16.7 per cent. (In this case the mice had been maintained on the diet for only two weeks.) The relative incidence of survival in the different groups of mice fed on a number of diets is tabulated below.

Effect of Variations in Diet on the Resistance of Adult Mice to the Virus of Yellow Fever (Kuczynski)

Diet*	Experimental Animals	Survivors, per Cent	Incidence of Gastric Ulcers, per Cent
Oats and milk	671	20	58
Oats and water	...	18.6	80
Canary seeds in milk	120	19	34
White bread and milk	...	25	20-30
Whole rye bread and milk	25	52	0
German rye bread and milk (87% rye flour, 8% wheat flour and 5% potato starch)	103	53	35
Whole wheat meal and milk	400	44	11-25
Knäckebröd† and milk	...	30.4	26
Knäckebröd† (from oatmeal) and milk	...	64	20-25
Whole wheat meal (extracted with alcohol) with milk	...	30	40-64
Wheat husks and milk	28	75	..
Wheat germ, potato and milk	25	57	..
Wheat germ, potato, yeast and milk	25	68	25
Egg yolk, white bread and milk	127	22	..
Edam cheese and milk	60	12	35
Edam cheese, rye bread and milk	28	60	18
Oats, milk and wheat oil	25	59	4
Oats, milk and olive oil§	54	72	18
Oats, milk and linseed oil§	24	87	0
Oats, milk and cod liver oil§	65	63	3
Casein and rice (free from vitamin B_1)#	137	16.7	68
Casein and rice (free from vitamin B_1), and yeast and whole rice	51	76.4	..

* All diets except when otherwise indicated had, in addition to the ingredients specified, butter fat and McCollum's salt mixture.

† Knäckebröd is a Swedish bread that is made up of whole meal of various cereals ground very finely and smoothly and rapidly baked in thin layers for from seven to eight minutes at a temperature of 100 C.

§ In this diet the butter fat was omitted.

This diet produced beriberi within a period of eighteen days and was therefore administered only for a period of fourteen days prior to inoculation with the virus.

Mice inoculated with the virus of yellow fever began to show ticlike twitchings within a few days, followed by spastic contractions of groups of muscles and eventually stupor with widespread motor paralysis lasting from a few hours to forty-eight hours, until death supervened. In those that survived, residual paralyses were observed, such as are seen in anterior poliomyelitis in human beings.

In mice that died, gastric ulcers were frequently present. Such ulcers reflected, apparently, injury to the adrenal cortex, and severe changes were observed histologically. The ulcers simulate those occurring after adrenalectomy or after severe burns. The incidence of the ulcers varied with the diets employed and depended not only on the vitamin B content but on the amount of roughage in the food. They were least frequent in mice fed diets containing oatmeal, milk and wheat oil.

All animals that survived one infection were again given the standard diet containing oats and milk and reinoculated with large quantities of emulsions of the virus. All proved to be immune. All the mice inoculated lost weight, and

sample mice killed on the fifth day after the first inoculation showed evidence of virus infection in the brain, as determined by injecting the emulsified brain substance into other mice.

Kuczynski stated that he obtained unfavorable results in mice that were fed diets composed of the following food constituents: (1) autoclaved rice, salt, milk and butter fat, in combination with small amounts of vitamin B_1 preparations; (2) mashed potatoes and milk; (3) beans, corn and milk; (4) carrots and milk. Mice fed on such diets succumbed in almost 100 per cent of the instances when inoculated with the yellow fever virus.

Kuczynski observed further that, though mice fed diets containing foods rich in vitamin B_1 demonstrated greatly increased resistance to the virus of yellow fever, this resistance was enhanced on addition of vegetable oils, such as peanut oil, olive oil, cottonseed oil or even linseed oil, to the diet in place of butter fat. The data on this increase in resistance do not appear as convincing as the experiments with variations in the additions of food rich in vitamin B.

It is justifiable to conclude from the extensive experiments of Kuczynski that mice which are fed diets adequate in the known necessary constituents and, in addition, rich in preparations containing vitamin B (particularly wheat products) are considerably more resistant to infection with the virus of yellow fever, injected intracerebrally, than are mice fed a standard diet.

Kuczynski observed certain similarities between the changes induced by yellow fever and those induced by vitamin B_1 deficiency; i. e., both conditions caused lymphoid hypoplasia, bradycardia, hypoglycemia and paralyses. He suggested that the virus infection depleted the store of vitamin B_1 and that certain of the symptoms observed in the course of the infection were due to deficiency of this vitamin.[67b]

The epidemiologic significance of defective nutrition in the spread of yellow fever in human beings is stressed by Kuczynski as a result of his experiments and on the basis of clinical observations culled from the literature and from his own experience.

The resistance of the nervous system to virus infections, he concluded, is dependent on the availability of an adequate amount of vitamin B_1 and certain lipids. On the basis of his data he assumed that vitamin B_1 is an important factor in resistance to poliomyelitis, as well, and suggested that a diet rich in this vitamin be administered to patients early in the course of this infection.

67b. Kuczynski considered the marked hypoglycemia observed in yellow fever to be a reflection of the deficiency of vitamin B_1, stating that it was due to the toxemia and starvation and not entirely due to the injury of the liver. The basis for this contention was entirely speculative.

The work of Kuczynski is of fundamental importance. The fact that there were a number of variables is somewhat confusing and detracts from the reliability of his conclusions. The mice used were not of the same strain but were derived apparently from a number of sources.

Further, there can be little doubt that the dosage of virus used could not have been adequately standardized, for emulsions of brain tissue from different mice infected with the virus of yellow fever must contain varying quantities of virus. Aliquot portions of such emulsions would not necessarily contain equal quantities of virus. Many other variations were thus introduced; for example, several modifications of the diets. Nevertheless, from the type of the experiments performed and the numbers of animals observed, the conclusion seems justified that foods rich in vitamin B_1 enhance the natural resistance of mice to an infection with yellow fever virus introduced intracerebrally. It would be of greater value if such an infection were induced by a route less drastic in its direct effects. Studies should be undertaken with other neurotropic viruses, which should be introduced intranasally into mice, guinea pigs and monkeys, to determine further the effect on resistance of a diet rich in vitamin B_1.

IX. EFFECT OF AN EXCESS OF VITAMIN B ON NATURAL RESISTANCE IN MAN AND THE THERAPEUTIC USE OF THIS VITAMIN COMPLEX

The clinical data on the beneficial effect of administering vitamin B in excess are scattered and fragmentary. It is only in very recent years that the optimal requirements have been investigated, and as yet there is no certainty as to the amounts of this vitamin complex needed in different age periods. It seems that the amount consumed in the usual diet of the average person represents a relative deficiency, since few foods are rich in vitamin B. A consideration of the content of this vitamin complex in ordinary foodstuffs at once suggests that there is a relatively scarce distribution of it in the usual diet. Certainly in pregnancy and in infancy the amount of vitamin B available in food is definitely inadequate for optimal conditions of growth and development of the newborn.

The subtle effects on cellular metabolism are at present difficult to evaluate, and the far-reaching influence of prolonged relative deficiency of vitamin B on natural resistance can only be suspected. As has been pointed out, vague gastrointestinal disturbances in infants, children and adults, certain afflictions of the skin and the trophic changes dependent on neuritic manifestations are probably due to this relative deficiency. To add to the difficulty in interpretation, vitamin B contains at least

three or four factors: B_1, the antineuritic factor, and B_2 (or G), a complex which consists of factor P-P, B_6, riboflavin, B_4, factor W and possibly others.

It was suggested by Reyher [68] that certain debilitated children with a tendency toward spasmophilia are strikingly benefited by administration of vitamin B in excess, in the form of extracts. In analyzing the histories of 24 children that had been prematurely born, he found that the mothers during pregnancy did not concern themselves with diet for reasons of economy or taste and that all cereals and most fruits were omitted. The children were underweight and did poorly, but with the addition of mixed cereals and yeast they improved considerably and gained rapidly.

It was pointed out by Summerfeldt [69] that the milled cereals are poor sources of the vitamin B factors and since 30 per cent of the caloric intake of the average diet of children is derived from such products, care must be taken with the selection of the remainder of the diet to supply optimal amounts of vitamin B. Only small amounts are present in milk, most meats, fruits and vegetables. In a preventorium he observed a series of 21 children between the ages of 6 and 13 years. The majority of these children had been in the preventorium for from one to two months before the observations were begun and had received the usual diet of milk, meat, eggs, vegetables and fruit. One group of 11 were given during a period of ten weeks 4 ounces (125 Gm.) daily of a special cereal mixture containing wheat germ and dried yeast. The rest continued to eat the routine diet. Children on the special cereal gained more rapidly. When the control group were then given the diet high in vitamin B, they too increased greatly in weight.

Similar reports by other observers on the vitamin B needs of infants and children clearly indicate that the optimal requirement is greater than has previously been thought. The experimental work of Sure [70] and the clinical studies of West,[71] Bloxsom,[72] Morgan and Barry,[73] Hoobler [74] and others have demonstrated beyond question that the pregnant mother requires large amounts of vitamin B for optimal health conditions in the infant and that only a small percentage of the intake of this vitamin complex (B_1) is secreted in the milk. The rate of growth of infants

68. Reyher, P.: Ztschr. f. Kinderh. **36**:134, 1923.
69. Summerfeldt, P.: Am. J. Dis. Child. **43**:284, 1932.
70. Sure, B.: (*a*) Science **66**:265, 1927; (*b*) J. Biol. Chem. **76**:685, 1928.
71. West, J. H.: Arch. Pediat. **46**:646, 1929.
72. Bloxsom, A. P.: Am. J. Dis. Child. **37**:1161, 1929.
73. Morgan, A. F., and Barry, M. M.: Am. J. Dis. Child. **39**:935, 1930.
74. Hoobler, B. R.: Am. J. Dis. Child. **40**:919, 1930.

fed brewers' yeast as a supplement to their diets from birth was 100 per cent greater than that of normally fed controls (Bloxsom [72]).[75]

These investigators recommended the use of vitamin B not only in polyneuritis but in nutritional edema, colitis, gastrointestinal atony and, because of the relation to carbohydrate metabolism, diabetes.

The relation of increased growth to optimal natural resistance is not clearly established, but these are in part, at least, associated. More definite data, however, are essential to demonstrate this relationship. It is assumed even in experimental work that the better nourished, heavier animal of the same age is capable of resisting greater quantities of toxic material parenterally introduced. The comparative quantities of poison or toxin to be administered to an individual animal in a specific experiment are usually calculated on the basis of body weight in any given species. Such assumptions of estimating the toxicity of a drug per unit of body weight are inferential and not well established.

A few papers have appeared on the use of an excess of vitamin B in the treatment of gastrointestinal disorders, particularly constipation (Murlin and Mattill [76]; Marks [77]; Montague [78]). Improvement in general health, bowel function and nutrition and disappearance of indigestion and cutaneous eruptions were frequently noted.

Children and adults suffering from herpetic stomatitis, aphthous stomatitis, herpes labialis, acute gingivitis and ulcerative stomatitis rapidly improved following the addition of vitamin B as yeast tablets to their diets (Gerstenberger [62]).

The treatment of furunculosis and acne with yeast has long been recommended, and it is now known that its value is due to the vitamin B content (Skillern [79]; Reeves [80]; Hawk, Knowles and Rehfuss [81]; Pfannenstiel and Scharlau [82]).

Reeves [80] treated 130 midshipmen on daily rations of yeast for several weeks. Of 45 that had suffered from boils, 30 were cured and

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75. Rats fed a diet free from vitamin B_1 but otherwise apparently complete may show complete freedom from polyneuritis. Slight growth occurs when crystalline vitamin B_1 is supplied in amounts as low as from 1 to 2 micrograms per day. Increase of the dosage of this vitamin, even up to approximately a hundred times the amount necessary to prevent paralytic symptoms, increases the gains in weight (Williams, R. R.; Waterman, R. E., and Keresztesy, J. C.: Science **81**:535, 1935).

76. Murlin, J. R., and Mattill, H. A.: Am. J. Physiol. **64**:75, 1923.

77. Marks, H. E.: M. J. & Rec. **135**:231, 1932.

78. Montague, J. F.: M. J. & Rec. **137**:314, 1933.

79. Skillern, P. G.: J. A. M. A. **57**:968, 1911.

80. Reeves, I. S. K.: New York M. J. **115**:637, 1922.

81. Hawk, P. B.; Knowles, F. C., and Rehfuss, M. E.: J. A. M. A. **69**:1243, 1917.

82. Pfannenstiel, W., and Scharlau, B.: München. med. Wchnschr. **77**:619, 1930.

continued well without recurrences. Similar results were reported in the treatment of acne vulgaris by Hawk, Knowles and Rehfuss.[81]

Pfannenstiel and Scharlau [82] observed remarkable effects of supplementing the diet with vitamin B on infections of the skin and furunculosis induced by injections of staphylococci in rabbits. Abscesses developing within forty-eight hours after the injection of these organisms healed within a period of less than two weeks, as compared with a period of four weeks in the case of rabbits fed a normal diet.

SUMMARY

Clinical data on the beneficial effect of vitamin B in excess in the diet are scattered. The vitamin B in the diet of the mother during pregnancy and of the child during infancy is inadequate for optimal growth and development of the newborn. Supplementing the diet with this vitamin complex increases the rate of growth and possibly the natural resistance of the host. Vitamin B in excess increases the natural resistance to staphylococcic infection in man and is useful in the treatment of furunculosis and acne.

The available data on the therapeutic use of vitamin B is concerned largely with its use in conditions consequent on relative deficiency. There are no available experimental data on the effect of an excess of this vitamin complex or of any of its components on resistance to infection or on the mechanism of immunity other than the work of Kuczynski on yellow fever in mice (see part VIII).

X. IMPORTANCE OF VITAMIN B_2 (OR G) IN METABOLISM AND RESISTANCE

Since the discovery of vitamin B_2 (or G) in yeast and its significance in the prevention of pellagra, this vitamin complex has been separated into distinct parts. It contains apparently a number of independent elements: the human antipellagra factor (P-P), the antidermatitis factor for the rat B_6), riboflavin, B_4 and factor W. To our knowledge there are no references in print to a direct study of the effect of vitamin B_2 (or G) on resistance, but a few words may be said on the significance of this vitamin complex and its importance in physiology.

The classic experiments of Goldberger and Lillie [83] established unequivocally that pellagra in human beings is due to deficiency of a heat-stable factor in vitamin B. Earlier studies of Goldberger on the prevention of pellagra showed that a factor in addition to protein was needed to prevent the disease, that this factor, P-P, did not need protein

83. Goldberger, J., and Lillie, R. D.: Pub. Health Rep. **41**:1025, 1926.

to be effective, that it was derived from beef and from dried aqueous extract of yeast and that it alone was essential for the prevention of pellagra.

Black tongue in dogs can be prevented by the P-P factor and is the analogue of pellagra in man. The source of this factor, dried yeast, can be heated for twenty-four hours in the autoclave at 15 pounds (6.8 Kg.) of pressure without destroying the antipellagric substance, though this heating completely destroys the antineuritic vitamin, B_1. The P-P factor does not permit growth in the rat even when it forms 40 per cent of a diet otherwise complete except for vitamin B_1. It is interesting that an extract of cornmeal rich in vitamin B_1 is not antipellagric.

Elvehjem and his associates [84] reported that canine black tongue produced by a diet of the Goldberger type is cured dramatically by administration of nicotinic acid. This was confirmed by Street and Cowgill.[85] Whether nicotinic acid is identical with the P-P factor is not yet established.

However, human pellagra has been treated and cured with amazing rapidity. Complete healing of oral lesions occurred within seventy-two hours. The drug is administered by mouth in amounts of from 60 to 500 mg. daily, or it may be given intravenously. It has certain unpleasant effects (Spies, Cooper and Blankenhorn [86]; Smith, Ruffing and Smith [87]; Fouts and associates [88]).

On the other hand, Cook, Clarke and Light [89] found that nicotinic acid or amide was not equivalent to the antidermatitis factors in the nutrition of rats (B_6 and ribroflavin) and that nicotinic acid had no supplementary effect when combined with concentrates from rice polishings. (Crystalline nicotinic acid was isolated from rice polishings by Funk.[90])

In rats a diet deficient in vitamin B_2 (or G) results in ophthalmia, loss of hair on the neck and trunk, dermatitis and dried incrustations of the ears, which appear within seven weeks. Inflammation of the tongue and mouth also occurs. The entire condition is readily cured in a short time by adding this vitamin complex to the diet.

In studying pellagra-like syndromes in the rat, Findlay [91] observed that rats fed on a basal diet containing caseinogen, rice starch, salt mixture and cod liver oil but deficient in vitamin B_1 and B_2 lived an

84. Elvehjem, C. A.; Madden, R. J.; Strong, F. M., and Wooley, D. W.: J. Am. Chem. Soc. **59**:1767, 1937.

85. Street, H. R., and Cowgill, G. R.: Proc. Soc. Exper. Biol. & Med. **37**:547, 1937.

86. Spies, T. D.; Cooper, C., and Blankenhorn, M. A.: J. A. M. A. **110**:289, 1938.

87. Smith, D. T.; Ruffing, J. M., and Smith, S. G.: J. A. M. A. **109**:2054, 1937.

88. Fouts, P. J.; Helmer, O. M.; Lepkovsky, S., and Jukes, T. H.: Proc. Soc. Exper. Biol. & Med. **37**:405, 1937.

89. Cook, C. A.; Clarke, M. F., and Light, A. E.: Proc. Soc. Exper. Biol. & Med. **37**:514, 1937.

90. Funk, C.: The Vitamins, Baltimore, Williams & Wilkins Company, p. 171.

91. Findlay, G. M.: J. Path. & Bact. **31**:353, 1928.

average of thirty-four days. Some acquired polyneuritis; all became weak and emaciated. If autoclaved yeast containing only the B_2 complex was added to the original diet, the average duration of life was thirty-seven days. Polyneuritis also occurred in this group. If a yeast extract free from vitamin B_2 but rich in vitamin B_1 was given, the average duration of life was seventy days and within fifty-three days lesions of the skin developed on the chest, neck and abdomen and later on the front paws and on the backs of the hindlimbs. The skin became red and swollen, the hair fell out, and desquamation was intense. Microscopically, these cutaneous lesions showed congestion of the small vessels of the cutis vera, swelling of the collagen fibers, infiltration with polymorphonuclear leukocytes and lymphocytes, active keratinization, parakeratosis and occasionally desquamation of the whole epidermis. Subsequently there followed in some instances atrophy of hair follicles. Ulceration of the tongue and buccal mucosa occurred. In addition, the gastric mucosa near the cardia became papillomatous and showed metaplasia to squamous cells. Atrophy of lymph tissue in general was prevalent.

Salmon, Hays and Guerrant,[92] in addition, described moist lesions of the skin in vitamin B_2-deficient rats. These were associated with vesicle formation and pruritus. With laceration of the lesions, hemorrhage and eschars developed. In the late stages, arthritis appeared in a few animals. Secondary infections, such as pneumonia, were common. Culture of the cutaneous lesions revealed a gram-positive coccus, the growth of which these workers [92] succeeded in inhibiting by adding to the culture medium small amounts of the P-P factor.

In addition to giving a detailed description of the cutaneous lesions, Thatcher, Sure and Walker [93] emphasized the inflammatory lesions in the stomach, which consisted of infiltration of the submucosa and of the tissues between glands by eosinophilic leukocytes and lymphocytes and formation of ulcers and abscesses.

Through the excellent work of Day, Langston and O'Brien [94] it has been established that cataracts, usually preceded by inflammatory changes in the lids and corneas, occur with regularity in young albino rats receiving a diet deficient in vitamin B_2 (or G). Changes in rats' eyelids were noted by Goldberger and Lillie [83] some years ago. Alopecia around the eyelids, with conjunctivitis and ophthalmia, were observed by Chick and Roscoe.[95] Opaqueness of the eyeballs, ptosis of the lids, lacrimation

92. Salmon, W. D.; Hays, I. M., and Guerrant, N. B.: J. Infect. Dis. **43**:426, 1928.

93. Thatcher, H. S.; Sure, B., and Walker, D. J.: South. M. J. **23**:143, 1930.

94. Day, P. L.; Langston, W. C., and O'Brien, O. S.: Am. J. Ophth. **14**:1005, 1931.

95. Chick, H., and Roscoe, M. H.: Biochem. J. **21**:689, 1927.

and conjunctivitis were mentioned by Salmon, Hays and Guerrant.[92] Similar changes in the eyes were recorded by Findlay [91] and by Sherman and Sandels.[96]

Day and his associates [94] placed healthy albino rats of the Wistar strain, 21 days of age, on a diet consisting of casein, salt mixture, butter fat, cod liver oil, corn starch and an alcoholic extract of rice polishings (deficient in vitamin B_2 or G but rich in vitamin B_1). The controls received in addition autoclaved yeast. Another group was placed on a diet deficient in vitamin A. Only in those on a vitamin B_2-deficient diet did cataracts develop. Early in the disease, the conjunctiva and cornea became inflamed, and a mucopurulent discharge appeared in the conjunctival sac. Cultures of the discharge revealed Staph. aureus, Brucella bronchisepticus and a few diphtheroids. Normal animals inoculated with the discharge did not show similar changes. In the vitamin-deficient rats the lids rapidly became hairless and swollen; the cutaneous surface was covered by a moist exudate. Microscopically, at this stage, the skin and subcutaneous tissues were infiltrated by polymorphonuclear leukocytes. The cornea was either clear or clouded, and superficial corneal vascularization was observed. The oncoming cataract was evidenced as a white reflex behind the pupil. The cataractous lens was swollen, and microscopically one saw lymphocytic and leukocytic infiltrates with new blood vessel formation directly under the epithelium in the anterior stroma. Interstitial keratitis became pronounced. The fibers of the crystalline lens degenerated, and the lens was converted into an amorphous mass. Morgagnian globules were present throughout. Of a series of 48 rats receiving the vitamin B_2 (or G) deficient diet, bilateral cataracts developed in a period of from sixty to eighty-seven days in all. In one of 3 rats which survived this time, a unilateral cataract developed.

Administration of vitamin B_2 (or G), while readily preventing the cataract formation, cannot cure the condition described. The blindness persists. However, the process may be arrested immediately by feeding autoclaved yeast, which is high in this vitamin complex.

Similarly, cataracts and intersitial keratitis developed in nearly 100 per cent of Norway gray wild rats deprived of vitamin B_2 (or G). The earliest cataract observed was seen after a period of forty-six days on the deficient diet. The average time was seventy-two days (Langston and Day [97]).

O'Brien [98] showed that the keratitis bore no relation to the formation of cataract. The earliest changes were represented by nuclear cataract

96. Sherman, H. C., and Sandels, M. R.: J. Nutrition **3**:395, 1931.
97. Langston, W. G., and Day, P. L.: South. M. J. **26**:128, 1933.
98. O'Brien, C. S.: Arch. Ophth. **8**:880, 1932.

or opacities in the anterior or in the posterior subcapsular layers or in the cortex. Proliferation of the subcapsular epithelium occurred and extended backward over the posterior aspects of the lens. With autolysis of the lens fibers, typical morgagnian globules appeared, and in the older amorphous masses cholesterol crystals were deposited (older cataracts).

In order to produce cataracts in rats, the deficient diets must be commenced at weaning. Otherwise, vitamin B_2 (or G) is stored in sufficient quantities to prevent rapid depletion. There is no uniformity in the lens changes. They may begin in the nucleus, the subcapsular zone or the deeper cortex, or as a diffuse process.

Day and his associates [94] concluded that vitamin B_2 (or G) deals with the nutrition of epithelial structures, since a deficiency of this vitamin complex shows its effects on hair, skin, mucous membranes and the crystalline lenses. The factor in vitamin B_2 (or G) deficiency of which leads to cataract formation was definitely identified as riboflavin (Day, Darby and Langston [99]). Recently Day and Darby [100] reported that the failure of some investigators to observe cataract formation as a result of deficiency of riboflavin may have been due to contamination of their caseins with riboflavin.[101] In experiments in which various casein preparations were employed in the vitamin B_2-free diet, cataracts formed in some instances among rats fed one type of casein, while rats fed other types of casein did not show cataract formation. Eluates from such caseins exhibited a greenish fluorescence characteristic of riboflavin when viewed under ultraviolet rays.

It would be of great interest to study experimentally the resistance of these ocular tissues to infection in animals deprived of riboflavin for a length of time less than that necessary to produce anatomic changes. Apparently, the interstitial keratitis and purulent conjunctivitis are due to an infection that readily occurs in these areas as a result of change in the epithelial structures, in somewhat the same manner as the xerophthalmia of vitamin A deficiency.

Györgyi [13] in 1934 and 1935 had shown that vitamin B_2 (or G) contained at least two distinct constituents, riboflavin and vitamin B_6, and that a deficiency of these two substances leads to distinct cutaneous

99. Day, P. L.; Darby, W. J., and Langston, W. C.: J. Nutrition **13**:389, 1937.

100. Day, P. L., and Darby, W. J.: J. Biol. Chem. **119**:xxii, 1937.

101. It is well known that a high proportion of lactose and galactose in the diet, up to 70 per cent, may cause cataract in rats, and galactose is the major etiologic factor in this type of cataract (Mitchell, H. S.; Merriam, O. A., and Cook, G. M.: J. Nutrition **13**:501, 1937). In such animals sugar has been found both in the blood and in the urine. The relationship, if any, between this type of cataract formation and that due to deficiency of riboflavin is not clear.

lesions in the rat. Chick, Copping and Edgar[102] in 1935 described these two kinds of cutaneous symptoms as follows:

(*a*) A symmetrical florid dermatitis with redness, swelling and edema, affecting first the pores, lips, ears and nose. This condition is similar to the specific type of dermatitis described by Györgyi and a pellagra-like condition in rats described by Goldberger and Lilly in 1926. Györgyi[13] found that vitamin B_6[103] prevented and cured this specific cutaneous lesion.

Since available literature concerning the production and cure of the florid type of dermatitis in the rat presents many inconsistent results and conflicting conclusions, a series of studies was initiated to determine the effect of such factors as the carbohydrate, fat and other constituents of the diet on the production of this nutritional disorder and the relative efficiency of certain dietary substances in curing it (Guerrant, Chornock and Dutcher[104]). The florid type of dermatitis was produced consistently in rats receiving diets containing dextrose or sucrose as the source of carbohydrate and, to a varying degree, in rats receiving diets containing corn starch or rice starch. The florid form of dermatitis was also produced quite regularly in rats receiving diets that were 10 per cent lard or 20 per cent hydrogenated cottonseed oil and less regularly in rats receiving a similar diet that was 8 per cent filtered

102. Chick, H.; Copping, A. M., and Edgar, C. E.: Biochem. J. **29**:722, 1935.

103. The vitamin is not precipitated by salts of lead, mercury or silver or by trinitrophenol. It is adsorbed on fuller's earth from acid solution, is precipitated by phosphotungstic acid and migrates toward the cathode on electrodialysis. It is inactivated by the action of benzoyl but not by the action of nitrous acid. It is soluble in ethyl alcohol but is not extracted from a concentrated aqueous solution by acetone, amyl alcohol or ether. T. W. Birch and P. Györgyi (Biochem. J. **30**:304, 1936) suggested that the vitamin does not contain a primary amino group but is of the nature of a base and possibly contains a hydroxyl group. Fat has a sparing action on the vitamin.

According to T. W. Birch, P. Györgyi and L. J. Harris (Biochem. J. **29**:2830, 1935), vitamin B_6 is distinct from the human antipellagric and the canine antiblack tongue factor, since the usual Goldberger diet for producing black tongue is highly effective in curing the "specific" dermatitis of rats, while canine black tongue and human pellagra develop in the presence of liberal amounts of vitamin B_6 (in the form of maize meal) yet are readily cured by liver extract containing (by rat assay) much less B_6.

Studies in which diets were used consisting more largely of purified materials indicated that, in addition to vitamin B_1 and riboflavin, both vitamin B_6 and the antiblack tongue factor are needed for normal nutrition in the dog. Maize is important in the black tongue-producing diets, not, as some have supposed, because it supplies a toxic agent but because it is deficient in the P-P factor, and yet it is a good source of vitamin B_6. In the absence of maize the dog may show vitamin B_6 deficiency, which may dominate the picture, instead of black tongue.

104. Guerrant, N. B.; Chornock, F., and Dutcher, R. A.: J. Biol. Chem. **119**:xlii, 1937.

butter fat. Yeast, ether-extracted yeast, white cornmeal, corn oil, wheat germ oil and linseed oil were effective in curing this form of dermatitis. Of the oils tested, wheat germ oil proved most effective.

(*b*) Shedding of the hair without swelling or inflammation and development of bald patches, especially over the head and back. The eyelids are often stuck together with a serous blood-stained fluid which exudes from the eyes and nostrils. This condition was described by Györgyi [13] as nonspecific and was cured and prevented by riboflavin. These experiments demonstrate the indispensability of riboflavin as well as of vitamin B_6 for the maintenance of a healthy condition of the skin. Attempts to cure these cutaneous conditions by administering riboflavin or a concentrate of vitamin B_6 respectively, were not always successful except when alcoholic extracts of wheat or maize were used as a source of B_6.

Copping [105] pointed out the significance of the deficiency of riboflavin in maize in relation to human pellagra. Many substances that prevent and cure pellagra, such as liver, milk, meat and yeast, are rich not only in vitamin B_6 but in riboflavin. Pure riboflavin, however, does not cure human pellagra, nor does it cure black tongue in dogs.[105a]

From these observations, it is suggested by inference that deficiency of riboflavin or of vitamin B_6 or of both definitely lowers the resistance of the epidermal tissue to infection. The constituents of vitamin B_2 (or G), i. e., P-P, B_6 and riboflavin, play an important role in the maintenance of a healthy condition of the skin.

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105. Copping, A. M.: Biochem. J. **30**:845, 1936.

105a. The inhibition of growth produced when either the riboflavin factor or the B_6 factor of vitamin B_2 (or G) is omitted from the diet of young rats led to investigation of the metabolism of the excised tissues of such animals (Muus, J.; Bessey, O. A., and Hastings, A. B.: Proc. Am. Soc. Biol. Chem. **8**:72, 1937). After eight weeks on the experimental diet, the oxygen consumption of the diaphragm and of the liver was measured by the Warburg technic on (1) normal controls on a balanced diet, (2) controls on the experimental diet with all the factors of vitamin B_2 added, (3) rats on a diet deficient in the riboflavin factor only, (4) rats on a diet deficient in the vitamin B_6 factor only and (5) rats deficient in both the riboflavin and vitamin B_6 factors.

Absence of the riboflavin did not influence the metabolism of the liver, but that of the diaphragm was 30 per cent above normal. On histologic examination, no abnormalitiy of the muscle was observed except greater concentration of the nuclei.

Absence of vitamin B_6 resulted in a decrease in the metabolism of the liver amounting to 24 per cent, whereas the metabolism of the diaphragm was normal. Histologic examination revealed fatty infiltration of the liver but no abnormality of the muscle save that of greater concentration of the nuclei.

When both riboflavin and vitamin B_6 were absent, the metabolism of the liver was decreased 24 per cent and the metabolism of the diaphragm was increased 35 per cent. Clinical and histologic changes of the same degree of severity appear earlier in this group than when either factor alone was absent. See also Györgyi.[13]

Riboflavin is a yellow-green fluorescent, water-soluble pigment widely distributed in plants and animals. Its formula is 6,7-dimethyl-9-(d'-1'ribityl)-isoalloxazine. Its chemical synthesis was described by Booher[106a] and was largely developed by Kuhn, Györgi and Wagner-Jauregg,[106b] and its chemical properties were determined by a number of investigators, including Karrer and Schopp.[106c]

Through its isoalloxazine nucleus it is related to, and possesses a combination of the chemical properties of, a substituted benzene, anazine dye and a pyrimidine. In addition, it possesses a ribityl side chain attached to the N atom in position G, which relates it to the pentose sugars.

The flavins represent stable oxidation-reduction systems. Acetylation, methylation or loss of the sugar-like side chain of riboflavin does not significantly affect the oxidation-reduction potential of the flavin. Riboflavin forms a phosphoric acid ester (riboflavin-5[1]-phosphoric acid) which combines with a protein to form a yellow oxidation enzyme. The yellow oxidation enzyme was discovered by Warburg and Christian[106d] and has been isolated in crystalline form by Theorell.[106e] The chemical and enzyme properties of the yellow oxidation enzyme have been reviewed by Theorell.[106f]

This enzyme is presumably present in every living cell and is concerned with the chemical reactions involved in the respiration of cells.

Animals maintained on diets deficient in riboflavin (as contained in vitamin B_2 or G) for a considerable time were more seriously ill than was apparent from the outward signs. They appeared on the verge of collapse for some time prior to death. This is a reflection of cellular anoxemia or asphyxia due to lack of an essential catalyst of cellular oxidation.

It would be reasonable to assume that a deficiency of this factor would interfere with the resistance of the cell to abnormal irritants and toxins, but experimental work in this field is at present lacking.

In studies on the respiration of tumor cells a low concentration of riboflavin has been reported in carcinoma tissues (Kuhn and associates[106b]).

The flavin content of organs cannot be greatly increased, for the administration of ten times the quantity in the diet increased only a little the concentration of yellow pigment in the liver. Normally riboflavin is excreted in the urine, but after a period of deprivation of vitamin B_2 (or G) no riboflavin is excreted in the urine of the rat, and growth ceases. In man the excretion per day reaches to from 800 to 1,200 micrograms per day.

Riboflavin is not toxic in excessive amounts. Mice can tolerate a thousand times the daily requirement.

In regard to the relation of riboflavin to the adrenal cortex, Laszt and Verzàr,[106g] in studying the effects of the addition of 0.02 per cent iodoacetic acid to a complete diet in rats, observed that growth was retarded and alterations in the bones, skin and blood occurred, with hypertrophy of the adrenal cortex. Riboflavin-phosphoric acid restored growth, but riboflavin itself was ineffective. Growth could be restored as well by the administration of an extract of adrenal cortex. They concluded that riboflavin must be phosphorylated before it can be

106. (*a*) Booher, L. E.: J. A. M. A. **110**:1105, 1938. (*b*) Kuhn, R.; Györgi, P., and Wagner-Jauregg, T.: Ber. d. deutsch. chem. Gesellsch. **66**:566, 1932. (*c*) Karrer, P., and Schopp, K.: Helvet. chim. acta **17**:735, 1934. (*d*) Warburg, O., and Christian, W.: Biochem. Ztschr. **254**:438, 1932. (*e*) Theorell, H.: ibid. **272**:155, 1934. (*f*) Theorell, H.: ibid. **278**:263, 1935. (*g*) Laszt, L., and Verzàr, F.: Arch. f. d. ges. Physiol. **236**:693, 1935. (*h*) Verzàr, F., and

(Footnote continued on next page)

utilized physiologically and that the adrenal cortex is essential for this phosphorylation. Verzàr and Laszt[106h] believed that celiac disease in human beings is similar to the toxicity from iodoacetic acid. In both the formation of riboflavin-phosphoric acid is inhibited. Rudy[106i] could not confirm Verzàr's work, for he noted that in rats poisoned with iodoacetic acid growth was restored with simple riboflavin as effectively as with riboflavin-phosphoric acid. Laszt and Verzàr[106j] reported that adrenalectomy interferes with the resorption of fat and that the resorption is restored to the normal level by administering either an extract of adrenal cortex or riboflavin-phosphoric acid.

SUMMARY

A deficiency of vitamin B_2 (or G) leads to localized alopecia (deficiency of riboflavin), diffuse dermatitis (deficiency of B_6), conjunctivitis, interstitial keratitis and cataract of the crystalline lens (deficiency of riboflavin) of the albino and of the wild rat. Eventually rats succumb to the effects of these deficiencies.

In dogs, a condition of black tongue, dermatitis and severe anemia is directly referable to a deficiency of the factor P-P. In man, pellagra has been definitely established as a disease due to lack of the P-P factor in the dietary. Apparently, vitamin B_2 (or G) is important in the metabolism and nutrition of epithelial tissue. Its importance secondarily in the maintenance of resistance to infection, particularly of such epithelial structures as the conjunctiva and the skin, can only be inferred. Direct experimental evidence in this field is completely lacking, but such data would be highly desirable. The investigation of this problem may prove of great importance in the fields of dermatology and ophthalmology.

From recent work it is apparent that vitamin B_2 (or G) contains, in addition to the recently described factors B_4 and factor W (important in growth), a factor B_6 (deficiency of which in rats leads to a specific florid dermatitis analogous to pellagra in man), a cutaneous factor, riboflavin (deficiency of which leads to alopecia, conjunctivitis, and keratitis and cataracts), and P-P_1 (the antipellagra factor). These three factors —riboflavin, B_6 and P-P—are apparently of vital importance in the maintenance of a healthy condition of the skin.

XI. INTERPRETATION OF THE ROLE OF VITAMIN B IN NATURAL RESISTANCE

An evaluation of the literature has suggested that vitamin B plays a role in natural resistance to infection; that absence or relative deficiency of this vitamin complex from the diet leads to deleterious effects on growth, on survival capacity in infants and on gastrointestinal function. Disturbances in the function of the nervous system may follow depletion

Laszt, L.: ibid. **237**:476, 1936. (*i*) Rudy, H.: Ztschr. f. angew. Chem. **49**:323, 1936. (*j*) Laszt, L., and Verzàr, F.: Biochem. Ztschr. **228**:35, 1936.

of vitamin B. An increase in the susceptibility of the subject to certain infections may follow, and this increase in susceptibility is probably an expression of impairment in metabolic activity. A great deal of work has yet to be done in this important field, in which the surface has hardly been touched.

It is also evident from our brief review that whatever may be said of the significance of vitamin B_2 (or G) in resistance is entirely inferential. Though its lack is associated experimentally and in man with dermatoses, with interstitial keratitis, with cataract formation, with conjunctivitis, with alopecia and probably with severe anemia, and though its function is intimately concerned with the nutrition of epidermal structures, its role in the defense mechanism has yet to be determined.

It may be well to discuss briefly those aspects of physiology which are concerned with vitamin B as far as they are known, and the manner in which the vitamin B factors are utilized, before considering how relative lack or absence of this vitamin complex may disturb the mechanism of resistance.

At present, vitamin B is held to consist of the following several factors:

1. Vitamin B_1. This is the antineuritic factor and in most animals is required for growth. Deficiency leads to disorders of the nervous system.

2. Vitamin B_2 (or G). This contains the human antipellagra vitamin (P-P), the antidermatitis factor for the rat (B_6) and riboflavin. It also is required for growth and may contain the extrinsic factor essential in the formation of the hemopoietic principle.

3. Vitamin B_3. Such a factor has been described by Williams and Waterman as essential for growth in pigeons.

4. Vitamin B_4. This factor, described by Reader, is presumably essential for the growth and normal health of rats.

5. Vitamin B_5. This factor, described by Carter, Kinnersley and Peters but not well established, is also essential for the growth of rats.

6. Factor W. Recently this was added to the list as a distinct factor of growth in rats, apart from B_4, B_6 and riboflavin.

ROLE OF VITAMIN B_1 IN PHYSIOLOGY

Through the observations of Eijkman,[1] Grijns,[2] Fraser and Stanton,[107] Braddon,[108] and Vedder and Williams[109] the existence in rice polishings of a substance that prevents beriberi was established.

107. Fraser, H., and Stanton, A. T.: Etiology of Beri-Beri, Studies from the Institute for Medical Research of the Federated Malay States, no. 10, Singapore,

(Footnotes continued on next page)

Since Williams in 1916 published the method by which he obtained a richer yield of vitamin B_1 from rice polishings, it has been possible to determine the chemical nature of this factor and to obtain it in crystalline form (Williams [110]; Windaus and his co-workers [111]).

The first synthesis was accomplished in 1936 by Andersag and Westphal [112] and Williams and Cline.[113] The synthetic product is already available as a stable hydrochloride under several trade names.

The substance is a combination of a pyrimidine base and a sulfur compound identified as a thiazole. It is neither oxidized nor destroyed by exposure to air.

Williams [110] derived from vitamin B_1, by treatment with liquid ammonia, a base, $C_6H_{10}N_4$, and from the pyrimidine–sulfonic acid derivative formed by cleavage of the vitamin with sulfite another base, $C_6H_9N_3$, identical with synthetic 2,5-dimethyl-6-aminopyrimidine. Williams and Cline [113] in August 1936 synthesized vitamin B_1 hydrobromide.

Of the synthetic crystalline material, 1 international unit is present in 2 micrograms (or 0.002 mg. of betaxin). The average need of the human being is estimated at from 150 to 300 international units a day.

Harris and Leong found, by a simple method of extraction from the urine (adsorption on acid clay), that healthy persons on a normal diet eliminate 20 international units of vitamin B_1 a day. They stated that a daily excretion of less than 12 international units is indicative of an inadequate intake of vitamin B_1 in the diet.

The symptoms in animals deprived of vitamin B_1 that are indicative of disordered function have already been discussed. McCarrison [26] wrote of two types of this disorder: polyneuritis columbarum and beriberi columbarum. Polyneuritis is associated with anorexia, asthenia and atrophy of most organs except the adrenals. Beriberi is characterized in addition by cerebellar symptoms, enlargement and dilatation of the heart, edema and ascites.

Kelly & Walsh, 1909; Collected Papers on Beri-Beri, Studies from the Institute for Medical Research of the Federated Malay States, no. 17, London, John Bale, Sons & Danielsson, Ltd., 1924.

108. Braddon, W. L.: Cause and Prevention of Beri-Beri, New York, Rebman Co., 1907.

109. Vedder, E. B.: The Prevention of Beri-Beri, Tr. Internat. Cong. Hyg. Demog. (1912) **5**:671, 1913. Vedder, E. B., and Williams, R. R.: Philippine J. Sc., s.B **8**:175, 1913.

110. Williams, R. R.: J. Biol. Chem. **25**:437, 1916; J. Am. Chem. Soc. **57**:229, 1935; **58**:1063, 1936.

111. Windaus, A., and others: Ztschr. f. physiol. Chem. **204**:123, 1932.

112. Andersag and Westphal, cited by Grewe, R.: Ztschr. f. physiol. Chem. **242**:89, 1936.

113. Williams, R. R., and Cline, J. K.: J. Am. Chem. Soc. **58**:1504, 1936.

Edema and dilatation of the heart have long been known to occur in beriberi in man. The edema is extrarenal, and there is no disturbance of salt and water metabolism. The effect on the heart and blood vessels may be indirectly due to the fact that vitamin B plays a part in the carbohydrate metabolism of muscle.

Wenckebach [114] described the cardiac symptoms in patients with beriberi in considerable detail. He noted: marked enlargement of the heart, murmurs that increased disproportionately with exercise; visible and palpable pulsations over the heart to the left of the sternum; a bounding pulse and thrill over the great arteries; overdistention of the veins of the neck and arms; marked dyspnea, and orthopnea. The electrocardiogram remained normal. Death sometimes occurred suddenly.[115]

Cowgill [40] observed that the effect of administration of vitamin B_1 on the heart of vitamin B_1-deficient pigeons is striking and that there is no true hypertrophy in beriberi. Diminution in size of the heart is associated with increase in the excretion of urine. The enlargement of the heart is due to edema fluid, as was definitely shown by Wassermeyer in B_1-deficient birds. It is probable that the general edema in beriberi may be secondary to heart failure.

Weiss and Wilkins [116] were of the opinion that dysfunction of the cardiovascular system resulting from lack of balance in the food intake is a disease of regular occurrence in the United States. They reported their studies in 120 such cases and presented evidence to indicate that deficiency of vitamin B_1 plays a primary role in the precipitation of the disease. They believe that the cardiovascular changes are dependent on changes in the nervous system, in the vascular system and in the myocardium. The clinical signs include tachycardia, followed by bradycardia, gallop rhythm, vagal reflex, irritability, dilatation of the heart, dyspnea, orthopnea and pulmonary congestion, associated with bounding arterial pulsation, arterial "pistol shot" sounds, engorged veins, warm skin and edema. These patients responded promptly to vitamin B_1 therapy. Following the use of 50 mg. of the crystalline product daily improvement was most rapid in some patients with severe congestive failure. Within twenty-four hours, there was increased utilization of oxygen, marked diuresis, slowing of the heart rate and decrease in the venous pressure.

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114. Wenckebach, K. F.: Lancet **2**:265, 1928.

115. The value of vitamin B_1 in the treatment of beriberi heart has been conclusively demonstrated by S. Weiss, R. W. Wilkins and F. W. Haynes (abstracted, Am. Heart J. **13**:503, 1937). An excess of vitamin B_1 of all types has been found to be of definite value in increasing cardiac reserve in the presence of cardiovascular disease (Sure, B., and Jones, W. A.: Proc. Am. Soc. Biol. Chem. **8**:93, 1937. Jones, W. A., and Sure, B.: J. Lab. & Clin. Med. **22**:991, 1937).

116. Weiss, S., and Wilkins, R. W.: Tr. A. Am. Physicians **51**:341, 1936; Ann. Int. Med. **11**:1, 1937.

Relation of Vitamin B_1 to the Processes of Oxidation and to the Nervous System.—Considerations of the nature and cause of the nervous manifestations have led to considerable controversy. Many investigators have attributed the disturbances to depression in oxidation, particularly in that of nerve tissue. There is some evidence that the oxidation of sugars in animals deprived of vitamin B_1 is incomplete. However, no significant fall in the consumption of oxygen by resting rats fed on diets deficient in vitamin B_1 has been observed until the period just prior to death of the animals (Drummond and Marrian[117]). Even in this period the reduction is attributed to inanition rather than to the deficiency of vitamin B_1. By measuring the utilization of oxygen and the ability to reduce methylthionine chloride under anaerobic conditions, no loss of oxidative activity in liver and muscle tissues were observed. On the basis of these findings, the importance of vitamin B_1 in the processes of oxidation was questioned.

However, Whipple and Church[118] recently reported respiratory quotients of less than 1 in vitamin B_1-deficient rats after subcutaneous injections of 1 Gm. of dextrose, respectively, while their isocaloric controls, receiving all the vitamins, showed quotients well above 1.

The administration of crystalline vitamin B_1 raised the average respiratory quotient of animals showing severe symptoms of beriberi from 0.79 to 1.40.[119] In this connection, the recent observations of Sure and Smith[120] and Sure and Buchanan[121] on the antithyrogenic action of crystalline vitamin B_1 are important. They demonstrated that retardation of the loss of weight can be effected in rats receiving doses of pure crystalline thyroxin by administering either a potent vitamin B concentrate or crystalline vitamin B_1. Pure crystalline vitamin B_1 is an antithyrogenic agent which in daily doses of from 30 to 100 micrograms

117. Drummond, J. C., and Marrian, G. F.: Biochem. J. **20**:1229, 1936.

118. Whipple, D. V., and Church, C. F.: Proc. Am. Soc. Biol. Chem. **8**:103, 1937.

119. However, vitamin B_1 may also be an essential constituent for the synthesis of fat in the body. Whipple and Church[118] observed that the tissues of rats deficient in vitamin B_1 contained less fat than the tissues of controls receiving an isocaloric intake and an adequate amount of vitamin. They expressed the belief that vitamin B_1 plays a role in the synthesis of fat from carbohydrate. In this connection, Salmon and Goodman (J. Nutrition **13**:477, 1937) observed that when the percentage of fat in the vitamin B-deficient diets of rats was high the rate of growth was increased and the incidence of beriberi decreased. The effectiveness of esters of fatty acids in alleviating the symptoms of vitamin B_1 deficiency depended on the length of the carbon chain of the fatty acid and was maximal at the 8 carbon atom fatty acid. Spastic beriberi in rats was cured by feeding glyceryl caprylate or caproate. The advantage of a diet high in fat over one low in fat seems to disappear when the diet contains sufficient vitamin B and the essential unsaturated fatty acids.

120. Sure, B., and Smith, M.: J. Nutrition **7**:147, 1934.

121. Sure, B., and Buchanan, K. S.: J. Nutrition **13**:513, 1937.

is able to counteract the toxicity of as much as 0.2 mg. of thyroxin. Peters,[122] however, noted a definite increase in lactic acid in the brains of pigeons, associated with symptoms of opisthotonos, in the terminal stages of B_1 deficiency; this increase was independent of the high level of sugar in the blood sometimes found. This suggested that in part the symptoms in the pigeons might have been associated with a disturbance of the oxidative mechanism of the brain or of certain areas of the brain, and such a disturbance may not be detectable by estimating the total amount of oxygen consumed in the whole animal (Kinnersley and Peters [123]).

In further experiments, this contention was substantiated, and it was found that additions of B_1 concentrates restored the oxygen consumption of the brain of the pigeon with beriberi almost to the normal level, while the lactic acid disappeared. The work was confirmed in experiments in vitro.

It was further established that this reduction in the oxidative activity of the brain is not due to inanition but is caused by the deficiency of vitamin B_1. Such nervous phenomena as are noted in birds with beriberi are not specific for B_1 deficiency but may be produced also by certain poisons, such as cyanides, that inhibit oxidative processes in the body.[124]

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122. Peters, R. A.: Biochem. J. **30**:2206, 1936.

123. Kinnersley, H. W., and Peters, R. A.: Biochem. J. **24**:711, 1930.

124. Rats fed a diet deficient in vitamin B show a marked increase in susceptibility to the effects of insulin. Whereas normal rats may withstand 200 units of insulin per kilogram of body weight, vitamin B-deficient animals are thrown into fatal shock by 20 units (McIntyre, A. R., and Burke, J. C.: Proc. Am. Physiol. Soc., 1937, p. 108).

W. B. Hess (Lancet **1**:100 and 554, 1932) studied in vitro the oxidative processes in different tissues of pigeons with beriberi. The animals were decapitated, the blood was washed out with ice-cold Ringer's solution to stop fermentative action and 1 Gm. of each organ was prepared with 10 cc. of Ringer's solution. To each portion 0.1 Gm. of m-dinitrobenzene was added, a thorough mixture made and oxygen excluded by nitrogen washing. The tubes were placed in a water bath at 40 C. for an hour. The centrifuged solutions were controlled by a photometric method, and values giving the measure of power of oxidative processes in the different tissues were determined on the basis of the reduction of m-dinitrobenzene to m-diphenylhydroxylamine. The oxidative processes were found reduced in every examined tissue of the vitamin B-deficient pigeons as compared with the corresponding tissues of healthy pigeons.

Hess further noted that in healthy pigeons intramuscular injection of potassium cyanide in doses of from 0.25 to 0.45 Gm. per hundred grams of pigeon weight resulted in all the classic symptoms of avian beriberi (sleepiness, ruffling of feathers, drunken swaying of the body on standing, dyspnea, nystagmus, opisthotonos, convulsions and paralysis).

Since cyanide inhibits oxidative processes, it was concluded that vitamin B_1 deficiency produces its effects through inhibition of oxidation. It was suggested that vitamin B_1 furnishes the structural basis of oxidative frements in the body.

Sherman and Elvehjem [125] determined the consumption of oxygen in normal and in polyneuritic chick tissues, using pyruvate as a respiratory substrate, and they observed that the respiration of the vitamin B_1-deficient cerebrum is subnormal. The ability of the chick kidney to utilize the pyruvate substrate is impaired in polyneuritis. If, however, small amounts of vitamin B_1 are added to the substrate, the uptake of oxygen in the avitaminous cerebrum and kidney is increased.

In another series of studies, they noted that anaerobic glycolysis readily takes place in tissues of polyneuritic chicks. They did not believe that there is any evidence that vitamin B_1 functions in anaerobic carbohydrate metabolism. Its action appears to be limited to the oxidative metabolism of carbohydrate intermediates.

This has been thoroughly substantiated, and is now well established, by the work of Peters and his associates [126]. It has been mentioned that they discovered that more lactic acid occurs in the brains of pigeons showing acute symptoms of beriberi. They also observed the presence of pyruvate, and it became apparent that the vitamin influences directly the oxidation of the pyruvate rather than the lactic acid or lactate. In the carbohydrate metabolism of brain tissue, dextrose is converted into lactate, and the lactate in the presence of a dehydrogenase and of a cytochrome (derived from oxygen and oxidase) is converted into pyruvate. The pyruvate is immediately oxidized in the presence of vitamin B_1 and then eliminated. Pyruvate is never present in the normal respiring brain in appreciable amounts but accumulates from lactate in the brain of the avitaminotic pigeon in the absence, but not in the presence, of vitamin B_1. The addition of vitamin B causes the disappearance of the added pyruvate. The pyruvate disappears in the avitaminous brain at death.

It is interesting that the pyruvate has been found in the blood of birds and rats deficient in vitamin B and recently by Platt and Lu [127] in the blood of patients with beriberi in the Orient. In the absence of vitamin B_1 the pyruvate accumulates in the brain, producing toxic symptoms. Vitamin B_1 is therefore essential for the development of energy from carbohydrate, which is necessary in the normal functioning of the nerve cells. Other types of interference with some stage of the metabolism of sugar may, therefore, produce analogous symptoms. This is true in asphyxia, overdosage with insulin, cyanide poisoning and chloroform poisoning.

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125. Sherman, W. C., and Elvehjem, C. A.: Am. J. Physiol. **117**:142 and 151, 1936.

126. Peters, R. A.; Rydin, H., and Thompson, R. H. S.: Biochem. J. **29**:53, 1935.

127. Platt, B. S., and Lu, G. D.: Quart. J. Med. **5**:355, 1936.

From the observations of Peters and his co-workers,[126] vitamin B_1 is essential in the oxidation system of cell metabolism.

Insulin-like properties were ascribed to vitamin B_1 by Lajos,[128] who found that the international standard preparation when injected together with dextrose into fasting rats caused increases in muscular and hepatic glycogen similar to those induced by insulin as well as some decrease in blood sugar. Vorhaus, Williams and Waterman[129] noted increased utilization of carbohydrate in some diabetic persons treated with large doses of vitamin B_1.

In a reconstruction of the problem of the function of vitamin B_1 in the oxidation of pyruvate acid in the brain, Peters[122] rejected the evidence against the conception that lactate is directly oxidized to pyruvate in the pigeon's brain as a preparatory stage to complete oxidation. He advanced the view that the action of this vitamin is related specifically to pyruvate oxidase in its aerobic reaction. The course of the reaction is represented as

$$\begin{array}{ccccc} \text{Lactate} + \text{O} & \rightarrow & \text{Pyruvate} + (\text{n})\ \text{O} & \rightarrow & \text{Oxidation Products} \\ \pm & & & \nearrow & \\ \text{Pyrophosphate} & & & \text{Vitamin } B_1 & \end{array}$$

The best substrate of demonstrating the catatorulin effect is pyruvate. The lactate and pyruvate oxidase systems can be separated at acid p_H values, and vitamin B_1 and pyruvate are necessary to insure stability of pyruvate oxidase at p_H 6.6.

Several papers have appeared on the measurment of the carbonyl compounds in the blood of B_1-avitaminotic pigeons and rats and in human beings with beriberi (Thompson and Johnson[130]; Platt and Lu[127]). The carbonyl compounds are measured by the bisulfite-binding power of the blood, and the major portion of the increase in bisulfite-binding substances in the blood of pigeons and rats deficient in vitamin B_1 is due to pyruvic acid.

An increase in the bisulfite-binding substances in the blood occurs in a number of diseases. Most prominent among these are diabetes with acidosis, febrile infectious diseases and organic heart disease with severe congestive failure, as well as frank vitamin B_1 deficiency (Taylor, Weiss and Wilkins[131]). It is known that febrile episodes occur in the course of beriberi and that symptoms of deficiency are aggravated by infections.

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128. Lajos, S.: Biochem. Ztschr. **284**:279, 1936.

129. Vorhaus, M. G.; Williams, R. R., and Waterman, R. E.: J. A. M. A. **105**:1580, 1935.

130. Thompson, R. H. S., and Johnson, R. E.: Biochem. J. **29**:694, 1935.

131. Taylor, F. H. L.; Weiss, S., and Wilkins, R. W.: J. Clin. Investigation **16**:833, 1937.

Taylor, Weiss and Wilkins observed a fairly close correlation between the elevation of the bisulfite-binding substances and the severity of the infection. The fever per se is not responsible. They expressed the belief that dehydration with reduced renal function and ketosis, as evidenced by ketonuria, play contributing roles. With recovery, the level of the bisulfite-binding substances returns to normal. The effect of vitamin B_1 in lowering the level of the bisulfite-binding substances in certain cases of vitamin B_1 deficiency suggests the important role of this vitamin in the processes of the oxidative metabolism.

The work of Peters and his associates [132] and others suggests a wide and fertile field for research on the possible etiologic role of vitamin B_1 deficiency in the susceptibility of animals to viruses specifically affecting nerve tissue. It is possible that its importance in resistance to encephalitis, poliomyelitis and other infections of the nervous system may be considerable, but as yet the problem has not been approached. (See yellow fever, p. 694.)

The effect of disturbances of peripheral sensory nerves on the resistance of the skin to infection has already been discussed. Trophic changes following a disease of the peripheral nerves result in alterations in the metabolism of the skin, with increase in susceptibility to injury and to bacterial invasion and decrease in the capacity to repair injuries of tissue.

The discussion of metabolic changes in vitamin B_1-deficient animals has been limited thus far to those observed in studies of the carbohydrate metabolism and respiration of the central nervous system and other tissues. The nitrogen, sulfur, sodium, potassium and chloride metabolism of rats deficient in vitamin B_1 was studied by Sandberg, Perla and Holly.[133] The only significant disturbance in the metabolism of these elements was that shown in the increase in the urinary excretion of nitrogen and of sulfur, which exceeded to a considerable extent the increase in the excretion of nitrogen and sulfur in the paired feeding controls.

The disturbance in protein metabolism, with a trend toward a negative nitrogen balance, is usually associated with alterations in natural resistance. Sandberg and Perla observed this in adrenalectomized [134] and in hypophysectomized rats.[135]

It is, however, striking that depletion in vitamin B_1 is not associated with disturbances in the metabolism of most of the essential elements

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132. Peters.[122] Peters and others.[126]

133. Sandberg, M.; Perla, D., and Holly, O.: Proc. Soc. Exper. Biol. & Med. **37**:350, 1937.

134. Sandberg, M., and Perla, D.: J. Biol. Chem. **113**:35, 1936.

135. Perla, D., and Sandberg, M.: Endocrinology **20**:481, 1936.

of the diet, though vitamin B_1 is so significant in oxidative processes in cells of the central nervous system and probably in cellular metabolism in general.

Of considerable interest is the work of Hamamoto [136] on the relation of vitamin B_1 to the metabolism of manganese. He found a pronounced decrease in the concentration of manganese in the tissues of pigeons deficient in vitamin B_1 and expressed the belief that manganese is essential in all oxidative processes in which vitamin B_1 is concerned. In part, some of the symptoms of vitamin B_1 deficiency can be delayed by increasing the manganese in the diet. Excessive manganese feeding has, however, a deleterious effect. If confirmed, this interrelationship between manganese and vitamin B_1 may be of fundamental importance.

A deficiency of vitamin B in the diets of pigeons and rats was found to impair the rate of healing and of scar formation in wounds of the skin, subcutaneous tissue and muscle (Saitto [137]). Saitto [137] expressed the belief that either administration of vitamin B by mouth or direct application of concentrated vitamin extract accelerated the rate of repair in normal rats. However, Lauber [138] found no increase in the rate of healing of wounds in animals receiving additions of yeast to their diet over controls fed the usual ration.[139].

Relation of Vitamin B_1 to the Health of Infants.—It is known that a partial deficiency of vitamin B in the diet of the pregnant mother will lead to a severe debilitating condition of the offspring.

Experimentally, in this regard, Sure [70a] showed that the optimal requirement of vitamin B varies with the condition of the mother and the age of the nursing young. Rats with litters of 6 which had been fed a diet containing adequate amounts of vitamin B in the form of extracts of wheat embryo were placed on a vitamin B-deficient synthetic diet for a period of twelve days. When the nursing young had ceased to increase in weight, the mothers were given brewers' yeast, but they required large amounts to wean their litters successfully. If the mothers of litters were given inadequate amounts of vitamin B, polyneuritis appeared in the litters. Small amounts of vitamin B given to the young produced rapid recovery, and the young then could be weaned

136. Hamamoto, E.: Orient. J. Dis. Infants **18**:21 and 57, 1935.

137. Saitto, S.: Scritti biol. **5**:273, 1930.

138. Lauber, H. J.: Beitr. z. klin. Chir. **157**:244, 1933.

139. G. Selfridge (Ann. Otol., Rhin. & Laryng. **46**:93, 1937) observed definite demyelination in both branches of the eighth nerve in a series of rats and chicks maintained on diets deficient in the different factors of vitamin B, especially the filtrate factors. He expressed the belief that these studies supported the contention that insufficiency of vitamin B in the human diet is probably the principal cause of the degeneration found in the eighth nerve. He was able to observe clinical improvement in hearing as determined by audiograms following the use of vitamin B_1 parenterally and of B_1B_2 adsorbate by mouth.

readily. If the vitamin was given to the mother during the suckling period, insufficient amounts were excreted in the milk to repair the effects of the previous deficiency in the young.

In further studies, Sure [70b] definitely established that the lactating mother (albino rat) does not eliminate adequate amounts of vitamin B_1 in the milk. Mortality in the young is high even when the mother receives as much as 1,500 mg. of yeast daily. This is due to the fact that the mother stores vitamin B_1 in her tissues and is unable to excrete vitamin B_1 (as furnished by yeast) quantitatively. Consequently, the young may die even though the mother regains her body weight. Rearing of the young is improved and infant mortality avoided if during lactation the young rather than the mother receive the yeast. For example, if the mother received 1,500 mg. of yeast daily when the young were 27 days old, the time required to wean the litter after this amount was introduced was sixteen days. During this period, the mother recovered 28 per cent of her weight. Weaning occurred in from four to five days when the daily dose of 1,500 mg. of yeast was taken from the mother and administered to the litter. The estimated minimal daily dose of yeast concentrate for the mother when all vitamin B_1 is expected to be transferred through the mother's milk to the young is 500 mg. Three hundred milligrams is inadequate for the young when given to the lactating mother, yet 180 mg. of vitamin B concentrate distributed between the mother and the young gives absolute success in rearing and weaning. The lactating mother wastes 60 per cent, if not more, of the dose of vitamin B in the metabolism of this vitamin complex before its passage into the milk.

Autopsy on the infant rats dying in those litters in which the distribution of vitamin B was inadequate showed hemorrhages in the intestinal tract, distention of the stomach and gastritis.

Maurer and Tsai [140] compared groups of female rats on diets adequate in, and low in, vitamin B content. They found that the mortality of the newborn was high in the group in which the mothers had been fed a diet deficient in vitamin B_1. The vitamin B_1 content was just adequate for maintenance of normal nonpregnant rats. The labor of these mothers was difficult and prolonged. Dead young were found frequently. The observations confirm, in general, the more extended studies of Sure [70] on the rat.

Perla [141] observed that though vitamin B_1 is essential for lactation and weaning, an excess of vitamin B does not shorten the period of lactation for the rat beyond that observed when adequate amounts of vitamin B_1 are added to the diet. The normal stock had yeast added

140. Maurer, S., and Tsai, L. S.: Illinois M. J. **61**:30, 1932.

141. Perla, D.: Proc. Soc. Exper. Biol. & Med. **37**:169, 1937.

to their diet in amounts equivalent to 2 international units of B_1 daily. When this quantity was administered to the mother for some time prior to gestation, the young were weaned in about three weeks. Additions of brewers' yeast in amounts equivalent to 50 international units of vitamin B_1 per animal per day caused a marked increase in the weight curve for several generations but did not shorten the period of lactation. Furthermore, newborn rats of the stock that was given an excess of vitamin B_1 for two generations, given daily subcutaneous injections of from 30 to 50 international units of synthetic vitamin B_1 did not show any perceptible difference in the time of weaning as compared with the young of the control normal stock. Sure's [70a] observations, however, were concerned with relative deficiencies of vitamin B_1 and demonstrate the essential importance of adequate amounts of this factor for normal lactation and growth of the young.

It is of interest that a great excess of vitamin B_1 under certain conditions of diet may prove detrimental. Perla [141] observed that an excess of yeast or of betaxin (B_1) will produce disturbance in lactation and loss of the nursing instinct, evident in the first generation but almost universal in the second generation, with consequent starvation of the young and high incidence of cannibalism.

The effect was more pronounced in animals fed B_1 concentrate adsorbates. Synthetic vitamin B_1 was somewhat less toxic in excessive amounts, but similar interference with lactation and the nursing instinct in the second and third generations occurred. Vitamin B_1 in amounts equivalent to from twenty to forty times the maintenance requirement seems to have an effect of interfering with the capacity of the mother to rear her young and with the nursing instinct similar to that of a relative insufficiency of this vitamin. The conditions under which this disturbance occurs are not as yet thoroughly defined. That it is not due merely to the action of vitamin B_1 is suggested by the fact that rats may be raised on a Sherman breeding diet with additions of 500 international units of this vitamin daily for several generations without their showing disturbance in breeding or lactation.[142] This should in no way discourage generous use of vitamin B_1 in the diets of human beings, as the difference between the optimal requirement and the amount which may prove toxic after several generations is considerable. The excess given to the rats would be equivalent to from 5,000 to 10,000 international units of vitamin B_1 per day for several generations.

The experiments of Sure are extremely important in throwing a new light on the mortality in infants in their first year of life. The diet of American nursing mothers is relatively deficient in vitamin B_1. It is suggested that a great proportion of the mortality associated with gastrointestinal disturbances and gastrointestinal infections in infants during their first year of life is caused by deficiency of vitamin B_1. The striking fact is that the nursing infant requires large amounts of vitamin B_1 daily during lactation. This is particularly true if the vitamin is supplied to the lactating mother and not directly to the infant, as most of it is not secreted in the milk.

142. Williams, R. R.: Personal communication to the authors.

On Pleasant Island in the Pacific, an English physician, Bray,[143] observed that the death rate of infants in the first year of life rose alarmingly with the substitution of European canned foods, lacking vitamin B_1, for a native mixture called toddy, which is rich in this vitamin. This rate was higher in the breast-fed infants. Breast milk is thought to be a perfect food and is known to be both antiscorbutic and antirachitic. However, suckling infants of mothers with beriberi in tropical countries acquire a disease which is due to deficiency of vitamin B_1. In this tropical island, frank beriberi did not occur in mothers, but the disease in infants was characterized by vomiting, distention and discomfort, irritability, intense cyanosis and convulsions. Death rapidly ensued, and only acute failure of the right side of the heart was observed at autopsy. This type of infantile beriberi may be complicated by pneumonia or meningitis and was most frequent in the third month of life. The duration of the disease was extremely short, death occurring within from twelve to twenty-four hours. The disease in some cases developed into a chronic form.

Large amounts of marmite, rice polishings, toddy, lactogen and other foods rich in vitamin B were given, and recovery took place in a few hours. Both the pneumonic and the meningeal form of the disease were due to congestion and edema and not to bacterial infection.

In the insidious forms in older artificially fed infants the disease was associated with loss of weight, digestive failure, subnormal temperature and lowered resistance to infection, shown in recurrent bronchitis, otitis media, pharyngitis or diarrhea. Leukopenia occurred. The organs showed acute congestion and passive hyperemia. There were also acute failure of the right side of the heart, degenerative lesions in nerve tissues and muscles and fatty degeneration of the liver and heart.

Beriberi in infants of mothers showing no clinical signs of disease is also observed in the Philippine Islands, Japan and other tropical countries where economic conditions of expectant or nursing mothers necessitate an inadequate diet, consisting almost entirely of rice. The disease is associated with gastroenteritis, vomiting, anorexia, loss of weight, pallor, irritability, scanty urination, colic and convulsions. The muscles are tender; the reflexes reduced. Often associated with these symptoms are infections. The disease is due probably to inadequacy and not to complete lack of vitamin B_1, for some antineuritic substances are present in the diet of breast-fed and bottle-fed babies in these countries. The intake of vitamin B in adults may be adequate except during pregnancy. The symptoms of infantile beriberi of this type completely disappear on addition of brewers' yeast to the diet.

143. Bray, G. W.: Tr. Roy. Soc. Trop. Med. & Hyg. **22**:9, 1928.

Maurer and Tsai,[140] in analyzing the death rate of infants in the state of Illinois, found that the drop in mortality observed in recent years was far less in infants under 1 week of age than in those who were older. Forty-four per cent of all infants dying during the first year of life die in the first week of life. The rural districts show a higher mortality of infants than the city districts. In places where mortality of infants is high, an analysis of the food shows that the diet of the mothers is low in vitamin B_1. Where consumption of this vitamin is high, the death rate of infants and the premature birth rate are low.

Goldberger's [12] analysis of the food of the working people shows that when food is plentiful it contains barely enough of vitamin B factors. During the winter months, inadequate amounts of these factors are consumed. Large numbers of Americans live on diets deficient in these factors during the winter, and this is the period of high mortality of infants. Feeding mothers large amounts of vitamin B during the last months of gestation will provide adequate storage of the vitamin B factors in the infants and will reduce the mortality of the latter in the first weeks of their life.

Though infections may occur in the newborn as a result of deficiency of vitamin B_1, they are apparently only incidental to the general metabolic disturbance.

The effect on the growth and nutrition of infants of supplementing an adequate dietary with vitamin B_1 was recently reported by Poole, Hamil, Cooley and Macy.[144] Their purpose was to determine whether the early addition of vitamin B_1 to an ordinary milk formula is advantageous to the nutrition and health of the average artificially fed healthy infant during the first twelve months of life. They observed 193 infants who at the onset of the experiment averaged 5½ weeks in age. The period of observation was one year. The infants were entirely artificially fed; 142 received supplements of B_1 in the form of rice polishings, in amounts equivalent to about from 40 to 50 international units per day (from 90 to 100 Sherman-Chase units); 51 received the same food formula without B_1 supplements. Monthly observations on growth, weight and general health were recorded. The bones were repeatedly examined roentgenographically, and blood counts were made at intervals.

The average daily gain in weight of the infants receiving supplements of vitamin B_1 was found to be more regular and consistent than the average gain of the controls. Group averages demonstrated fewer erratic fluctuations for those receiving supplements of vitamin B_1.

The authors believed that the vitamin B_1 added to the diets of 142 infants promoted stability of growth and of nutrition. There was little

144. Poole, M. W.; Hamil, B. M.; Cooley, T. B., and Macy, I. G.: Am. J. Dis. Child. **54**:726, 1937.

difference, however, in the time of appearance of the deciduous teeth in the treated infants and the controls, and development as demonstrated by walking, saying words and like performances did not appear to be accelerated by increasing the vitamin B_1 in the diet. Also, infections of the respiratory tract were not reduced in number in the treated group.

It is difficult to determine the vitamin B_1 requirements of human beings on the basis of clinical studies of balance, owing to inability to determine chemically the excretion of vitamin B_1. Biologic tests for B_1 in the urine, while feasible, have the disadvantage of variability in the determination of B_1 unitage. In a preliminary study Helmer [145] showed that the urine of an ordinary person contains demonstrable amounts of vitamin B_1. Harris and Leong,[146] in an extensive study of the daily excretion of vitamin B_1 in urine, utilized the bradycardia method of determining vitamin B_1 potency in rats. Their results showed that healthy adults varying in age from 17 to 37 years and on normal diets excreted daily from 12 to 35 international units or from 30 to 90 micrograms of vitamin B_1. From this study it was concluded that a urinary output of vitamin B_1 below 12 international units per day, or approximately 1 international unit per hundred cubic centimeters, may be considered as evidence suggesting inadequacy of vitamin B_1 in the diet. In children the average total daily output of vitamin B_1 as calculated on the basis of body weight is of the same order as in adults. As a result of these studies it was calculated that the minimum standard need of vitamin B_1 in a man weighing 140 pounds (63.5 Kg.) is about 200 international units.

It is known from the work of Cowgill [40] and of Vorhaus, Williams and Waterman [129] that the vitamin B_1 requirement of animals is related to their caloric intake. Cowgill was able to show, from studies on the minimal vitamin B_1 requirements of the rat, mouse, pigeon and dog, a correlation between the vitamin B_1 requirement per unit of body weight and metabolism, and he suggested that the relation of vitamin requirement to caloric intake and body weight might be expressed in general as follows:

$$\frac{\text{Vitamin (daily requirement vitamin B)}}{\text{Calorie (daily total energy exchange)}} = K \text{ (species constant)} \times W \text{ (body weight in grams)}.$$

Vitamin B is expressed in milligram equivalents (1 international unit equals 20 milligram equivalents), and K for the human species is 0.0000284.

It was found from a study of the vitamin content of human dietaries, some of which were known to have permitted the occurrence of beriberi, that the theoretical prediction of the formula for adequacy of the intake of vitamin B_1 was in agreement with the facts. For complete protection the vitamin-calory ratio was calculated to be more than 2. A liberal vitamin requirement in an adult with an intake of from 2,500 to 3,000 calories would be about from 300 to 375 international units. This ratio indicates a requirement of 12 international units per hundred calories. If this is correct, it is apparent that borderline deficiency of vitamin B_1 must be far more prevalent than is generally realized. That this amount of vitamin B_1 represents only slightly more than the minimal requirement is suggested from recent analyses of large numbers of dietaries from regions where beriberi is prevalent (Baker and Wright [147]). The vitamin B contents in these diets fell in the range

145. Helmer, O. M.: Proc. Soc. Exper. Biol. & Med. **32**:1187, 1935.
146. Harris, L. J., and Leong, P. C.: Lancet **1**:886, 1936.
147. Baker, Audrey Z., and Wright, Margaret D.: Brit. M. J. **1**:599, 1936.

of from 70 to 180 international units per day. Daniel and Munsell [148] stated that protective diets might vary in vitamin B from 145 to 500 international units, depending on the weights of the subjects. Baker and Wright [147] emphasized that there is evidence that children need more vitamin B_1 on the basis of caloric intake. The diet recommended by the Ministry of Health for Great Britain contains 450 international units for about 2,700 calories per child. It may be stated, then, that the vitamin B_1 requirement of the normal adult varies from 0.5 to 1 mg. of crystalline vitamin B_1 per day. Rose [149] feels that pregnant women should have double the quantity of vitamin B_1 that the normal adult needs, while nursing mothers should have triple this amount.

It is possible on the basis of the studies mentioned to obtain in what is generally considered an adequate dietary the necessary requirement of vitamin B_1 both for the adult and the child. There is evidence that various dietaries used in both the United States and Great Britain which are adequate in other respects may contain approximately from 200 to 400 international units of vitamin B_1 per day per person.[150]

Particularly high in vitamin B_1 are nuts, unpolished rice, soy beans, wheat bran, wheat germ, germinating cereals, beans, corn and pork. It must be remembered, however, that vitamin B_1 is unfavorably affected by high temperatures and thus much of its value may be lost in cooking.

ROLE OF VITAMIN B_2 OR (G) COMPLEX IN THE FORMATION OF HEMOGLOBIN AND GASTRIC SECRETION

From the observations of Whipple and Robscheit-Robbins [151] and those of Minot [152] and Minot and Murphy,[153] the specific effect of liver therapy in pernicious anemia was established. Subsequently, Castle and his co-workers [154] demonstrated the primary role of gastric disorders in the pathogenesis of the disease. They showed that remissions could be produced by administering beef muscle that had been digested with gastric juice. Neither the beef nor the gastric juice was effective alone, nor were the two when fed separately. Hydrochloric

148. Daniel, Esther P., and Munsell, Hazel E.: Vitamin Content of Foods: A Summary of the Chemistry of Vitamins, Units of Measurement, Quantitative Aspects in Human Nutrition and Occurrence in Foods, Miscellaneous Publication 275, United States Department of Agriculture, 1937.

149. Rose, M. S.: The Foundations of Nutrition, ed. 2, New York, The Macmillan Company, 1933, p. 630.

150. A comprehensive survey of the vitamin content of various foods is given in the bulletin of Daniel and Munsell [148] published by the United States Department of Agriculture.

151. Whipple, G. H., and Robscheit-Robbins, F. S.: Am. J. Physiol. **72**:395, 1925.

152. Minot, G. R.: Tr. Am. Neurol. A. **57**:329, 1931.

153. Minot, G. R., and Murphy, W. P.: J. A. M. A. **89**:759, 1927.

154. Castle, W. B.; Heath, C. W., and Straus, M. B.: Am. J. M. Sc. **182**:741, 1931.

acid, pepsin and rennin were eliminated as factors unnecessary in this action. Castle [155] suggested that pernicious anemia is a disease caused by lack of a substance produced from protein during the course of normal digestion. In the healthy person an extrinsic factor derived from the diet, present in muscle and protein in character, forms together with an intrinsic factor, a heat-stable antianemic principle, which is stored in the liver, kidneys and possibly other organs. Further work by Morris and his co-workers [156] demonstrated that simple neutralized gastric juice of swine, man and dogs contains the active intrinsic principle, that it is heat labile, that it is dialyzable through a collodion sac and that it withstands chemical treatment known to destroy enzymes. It is considered in the nature of a hormone normally secreted internally by the stomach. It may be, as suggested by Fouts, Helmer and Zerfas,[157] that gastric juice contains both the intrinsic and the extrinsic factor, which interact to form a hemopoietic agent.

Wills [158] reported instances of tropical macrocytic anemia cured by an autolyzed yeast product, marmite, rich in vitamin B_2. It was also used in treating a celiac disease together with a macrocytic anemia associated with the presence of free gastric acid. Strauss and Castle [159] expressed the belief that the extrinsic factor in the hemopoietic principle is closely related to vitamin B_2. The suggestion was advanced that the celiac disease, tropical macrocytic anemia and sprue that responded to yeast added to the diet had been due to a deficiency of the extrinsic factor and that the intrinsic factor was still present in the gastric juice. (Liver extracts and the muscle preparations of Castle are rich in vitamin B_2.) However, Wills [158] did not obtain the results with dried brewers' yeast or egg white, richer in vitamin B_2 than marmite, that she obtained with marmite, which is an autolyzed extract made from brewers' yeast. She held that the extrinsic factor is probably not vitamin B_2 but some other substance present in both animal protein and marmite.

Wills and Stewart [160] produced macrocytic hyperchromic anemia in monkeys by feeding them a diet in common use among Mohammedans of the poor class in Bombay, who suffer more severely than any other

155. Castle, W. B.: Am. J. M. Sc. **178**:748, 1929.

156. Morris, R. S., and Schiff, L.: J. Med. **13**:27, 1932. Morris, R. S.; Schiff, L.; Berger, G., and Sherman, J. E.: Am. J. M. Sc. **184**:778, 1932; J. A. M. A. **98**:1080, 1932.

157. Fouts, P. J.; Helmer, O. M., and Zerfas, L. G.: Am. J. M. Sc. **187**:36, 1934.

158. Wills, L.: Brit. M. J. **1**:1059, 1931; Indian J. M. Research **21**:669, 1933; Lancet **1**:1283, 1933.

159. Straus, M. B., and Castle, W. B.: J. A. M. A. **98**:1620, 1932.

160. Wills, L., and Stewart, A.: Brit. J. Exper. Path. **16**:444, 1935.

group in the city from tropical macrocytic anemia. The diet is low in protein and contains none of animal origin, and, though supplemented with vitamins A, C and D, it is very low in vitamin B, owing to the poor quality of the cereals and the absence of meat, liver and yeast from the diet. The monkeys were fed a diet consisting of polished rice, margarine, common salt, white bread, cod liver oil and tomato or carrot. They gradually lost weight and began to show anemia and edema, which were apparently related to the deficiency of vitamin B in the diet. The anemia was both macrocytic and megaloblastic with a high color index. The van den Bergh test, both direct and indirect, was negative, and there was no increased fragility; i. e., there was no increased destruction of blood, and the anemia was clearly a deficiency disease. It was cured promptly by additions of marmite to the diet.

Apparently, then, there is a factor in yeast which may be related to vitamin B_2 (or G), i. e., the essential extrinsic factor in normal hemopoiesis, and its absence from the diet leads to severe macrocytic hyperchromic anemia analogous to pernicious anemia in man.

Kyer and Bethell [161] observed the role of vitamin B in the prevention of nutritional anemia in white rats. Rats were given a basal diet of dry milk of known iron and copper content. Supplements of a 70 per cent alcoholic extract of dried brewers' yeast had a definite influence on the prevention and on the cure of anemia. The iron and copper content of the small amounts of yeast extract used could not account for this effect. Neither vitamin B_1 nor riboflavin had any effect, but vitamin B_4 exerted the same influence as whole yeast extract. They expressed the belief that vitamin B_4 stimulates erythropoietic activity in the white rat, possibly by influencing the synthesis of hemoglobin.

Day, Langston and Shukers [162] observed severe leukopenia and anemia and later ulcerative gingivitis and death in monkeys fed a diet deficient in vitamin B_2 (or G). The diet was adequate in respect to protein, inorganic elements, fat and vitamins A, B_1, C and D but deficient in vitamin B_2 (or G) and deficient in other less well known organic substances which may be essential to nutrition in the monkey. The foods used, with their percentages, were: casein, 10; whole wheat, 15; polished rice, 30; cod liver oil, 3; salt mixture, 4; sodium chloride, 2, and orange juice, 4. After a varying period of time on the diet, the animals presented a fulminating fatal blood disease characterized by anemia and leukopenia. The red cell count dropped to 3,000,000 or less from between 5,000,000 and 6,000,000, and the hemoglobin fell from 7 to 2.27 Gm. per hundred cubic centimeters of blood. The leukopenia

161. Kyer, J., and Bethell, F.: J. Biol. Chem. **109**:1, 1935.

162. Day, P. L.; Langston, W. C., and Shukers, C. F.: J. Nutrition **9**:637, 1935.

was moderate, the white cell count reaching 5,000 or less. Additions of dried brewers' yeast prevented the development of this deficiency syndrome.

The literature has a number of references to the influence on gastric secretion of a diet deficient in vitamin B. Danysz and Koskowski [163] and Farnum [164] reported marked decrease in both the volume and the acidity of gastric secretion in pigeons fed polished rice. Cowgill and Gilman [32] observed the development of gastric achlorhydria in dogs fed diets deficient in vitamin B. Webster and Armour [165] produced complete anacidity in dogs by giving them an avitaminotic diet and restored the gastric secretion with autoclaved or powdered yeast. They believed that the vitamins contained in yeast were absolutely essential to the normal secretory activity of the gastric mucosa. The syndrome which Miller and Rhoades [166] produced in dogs with feedings deficient in vitamin B_2 (or G) (a modification of Goldberger's diet for producing black tongue) resembled clinical sprue, with glossitis, anorexia, vomiting, diarrhea, loss of weight and severe anemia. It is known that pellagra, sprue and pernicious anemia all are characterized by anacidity, atrophic glossitis and gastrointestinal manifestations.

It is probable, therefore, that some factor in the vitamin B_2 (or G) complex may play a specific role in regulating gastric secretion and the formation of the hemopoietic factor.

There is often a reciprocal relationship between beriberi and diarrheal conditions. Thus, beriberi frequently occurs in the Orient with dysentery or ancylostomiasis. As a consequence of such diseases, the patient is unable to absorb sufficiently large amounts of vitamin B for bodily needs, and this failure of absorption contributes to precipitate the deficiency diseases. This hypothesis is unsubstantiated by experimental proof.

Depression in motility of the alimentary tract is a common manifestation of mild insufficiency of vitamin B. Deficiency of vitamin B_1 may lead to disturbances of the normal barrier to bacterial invasion of the intestinal tract by decreasing the secretion of gastric juice, by interfering with normal peristalsis and by producing gastric ulcers. (In certain instances mucous and ulcerative colitis are improved by supplementing the diet with vitamin B.)

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163. Danysz, M., and Koskowski, W.: Compt. rend. Acad. d. sc. **175**:54, 1922.

164. Farnum, M. B.: Arch. Int. Med. **37**:212, 1926.

165. Webster, D. R., and Armour, J. C.: Proc. Soc. Exper. Biol. & Med. **30**:1297, 1933.

166. Miller, D. K., and Rhoades, C. P.: Proc. Soc. Exper. Biol. & Med. **30**:540, 1933.

XII. GENERAL SUMMARY

In rats with deficiency of vitamin B the bactericidal power of the blood for Staph. aureus is definitely diminished. The titers of other natural antibodies are unaffected. However, in rats and pigeons, deficiency of vitamin B may lead to depression in the formation of agglutinin if dead organisms are used as antigen. The effect of deficiency in vitamin B alone in the bird has not been determined. When the diet has been more carefully controlled, deficiency of vitamin B does not impair the formation of specific antibodies.

Though further investigation is desirable, there is evidence that pigeons, rats and dogs fed diets complete in all known factors except vitamin B complex show decrease in natural resistance to spontaneous bacterial infections, such as S. suipestifer in the fowl, and to infections with a filtrable virus inducing a tumor-like condition, such as epithelioma contagiosum, in the pigeon. Rats fed diets deficient in vitamin B_1 acquire gastric ulcers. Dogs similarly fed show ulcerations and infections of the skin over the bony prominences of the legs, analogous to decubiti. Such disturbances are probably secondary to trophic changes precipitated by degenerative processes in peripheral nerves.

Though requiring confirmation and more extended observation, scattered evidence reported in the literature indicates an increase in frequency of spontaneous infections in animals and man fed diets deficient in vitamin B. This is dependent not only on alterations in cellular metabolism but also on trophic changes secondary to the induced polyneuritis.

Attempts to determine quantitatively the decrease in natural resistance by studying artificially induced infections or by administering toxic drugs to animals deficient in vitamin B are reported in a number of communications in the literature. The greater number of these reports do not stand critical analysis, for either the data are statistically inadequate or, more frequently, there is an indication that deficiency of other dietary factors was permitted. Particularly true is this of work in pigeons with beriberi induced by the feeding of a diet of polished rice. The results under such experimental conditions may be attributed to partial starvation or to complete avitaminosis, as well as to deficiency of vitamin B.

In pigeons, dogs and rats deficiency of vitamin B lowers the natural resistance to certain subsequently induced acute and chronic bacterial and helminthic infections. Mice suffering from deficiency of vitamin B, though more susceptible to induced infestation with hookworms than animals normally fed, show little alteration in response to mouse typhoid, nor do rats demonstrate a significant drop in resistance to infection with T. equiperdum under a similar dietary régime. The difference in results obtained with various infections is in part due to the fact that

certain bacteria synthesize vitamin B_1 in their metabolism, which may be available to the infected host. Some protozoa, such as T. equiperdum, may require vitamin B_1 for their growth and do not thrive in an environment depleted of this vitamin. Though the explanation of these apparent inconsistencies is not entirely clear, it is probable in spite of them that the vitamin B factors are essential in the maintenance of natural resistance to certain infections in birds and many mammals.

Leprosy in man occurs endemically in those geographic areas and countries where the diet of the population is particularly deficient in vitamin B. Experimentally, rats fed a diet deficient in vitamin B_1 but adequate to maintain suboptimal growth and reproduction show marked depression in natural resistance to rat leprosy in the third and fourth generation.

Mice fed diets adequate in the known necessary constituents and rich in preparations containing vitamin B, particularly the wheat products, are considerably more resistant to an induced infection with the virus of yellow fever, injected intracerebrally, than are mice fed a standard diet.

The clinical data on the beneficial effect of vitamin B in excess in the diet are scattered. The vitamin B content of the dietary in pregnancy and in infancy is inadequate for optimal growth and development of the newborn. Supplementing the diet with vitamin B increases the rate of growth and possibly the natural resistance of the host. Vitamin B in excess may increase the natural resistance to staphylococcic infection in man and is useful in the treatment of furunculosis and acne.

Deficiency of vitamin B_2 (or G) leads to localized alopecia, diffuse dermatitis, conjunctivitis, interstitial keratitis and cataract of the crystalline lens in the albino and in the wild rat. Eventually, the rats succumb to the effects of this deficiency.

In dogs, black tongue, a dermatitis and severe anemia are directly referable to deficiency of vitamin B_2 (or G). In man, pellagra has been definitely established as a disease due to lack of the P-P factor of this vitamin complex in the dietary. Apparently this vitamin complex (P-P factor, B_6 and riboflavin) is important in the metabolism and nutrition of epithelial tissue. Its importance secondarily in the maintenance of resistance to infection, particularly of such epithelial structures as the conjunctiva and the skin, is secondary and can only be inferred. Direct experimental evidence in this field is completely lacking but is highly desirable.

The role of vitamin B in natural resistance to infection is an indirect one and is secondary to its importance in cellular metabolism and the processes of oxidation. The physiologic activity of vitamin B_1 has been discussed in an attempt to correlate it with the mechanism of natural resistance. Vitamin B_1 is essential in the processes of oxida-

tion in brain and nerve tissue. It plays a significant role in growth, particularly in the young. It is essential for normal gastrointestinal peristalsis and gastric secretion. Some fraction of vitamin B, possibly related to vitamin B_2 (or G) is important in the formation of the antianemic principle and in hemopoiesis. Part of vitamin B_2 (or G) is concerned in the metabolism of epithelial tissue, of the skin and of the lens. One fraction of this complex, known as B_6, prevents certain cutaneous lesions in rats. Another fraction, riboflavin, prevents alopecia and formation of cataracts in the rat. A third factor, P-P, prevents pellagra in man. Riboflavin is essential in the synthesis of the yellow enzyme of Warburg, which is significant in cellular oxidation respiration.

Absence or relative deficiency of vitamin B_1 in the diet may depress the natural resistance of the nervous system to infection by impairing the consumption of oxygen. Trophic changes may occur in tissues supplied by peripheral nerves that have undergone degenerative changes incident to the polyneuritis associated with deficiency of vitamin B_1. In this manner, the natural resistance of tissues, such as the skin, to infection may be markedly depressed.

Deficiency of vitamin B complex may lead to disturbances of the normal barrier to bacterial invasion of the intestinal tract by decreasing the secretion of gastric juice, by interfering with normal peristalsis and by producing gastric ulcers. It may result in anoxemia of vital tissues by impairing hemopoiesis and thus interfering with the normal oxygen-carrying power of the erythrocytes. Anoxemia decreases the resistance of tissues to infections and toxins.

The role of vitamin B in the mechanism of natural resistance is dependent on its physiologic importance in cellular oxidative processes. Essential in the processes of growth, in the respiration of nerve tissue, in the maintenance of normal gastric secretion and intestinal tone, in hemopoiesis and in the metabolism of epithelial structures, its importance in the mechanism of natural resistance is suggested by inference. Direct experimental evidence and empirical observation suggest the significance of vitamin B in the physiology of natural resistance. However, a definition of the relative importance of the various factors of this vitamin complex in resistance is at present not possible.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Harvey S. Thatcher, professor of pathology at the University of Arkansas, died Jan. 20, 1938, at the age of 52 years.

Paul Ernst, professor of pathology at the University of Zurich from 1900 to 1907 and at the University of Heidelberg from 1907 to 1928, has died, at the age of 78 years.

A. N. Richards, professor of pharmacology at the University of Pennsylvania, received on March 9 the Philadelphia Award in recognition of his work on kidney action. The award, established by Edward W. Bok, consists of $10,000, a gold medallion and an engrossed scroll, and is conferred on a person "living in Philadelphia, its suburbs or vicinity, who, during the preceding calendar year, shall have performed or brought to its culmination an act or contributed a service calculated to advance the best and largest interests of the community of which Philadelphia is the center."

Peyton Rous, a member of the Rockefeller Institute for Medical Research, has been elected a corresponding member of the Academy of Medicine in Paris.

George W. McCoy, medical director of the United States Public Health Service, has been assigned, at the request of Dean Joseph Rigney D'Aunoy, to the school of medicine of the Louisiana State University. His title will be that of professor of preventive medicine and public health.

Edgar H. Norris, assistant in pathology at the University of Minnesota, has been appointed professor and head of the department of pathology at Wayne University College of Medicine, Detroit.

The Cameron Prize of the University of Edinburgh for 1938 has been awarded to Karl Landsteiner, a member of the Rockefeller Institute for Medical Research, for his researches on blood groups.

The Charles Mickle Fellowship of the University of Toronto has been awarded to George H. Whipple, of the University of Rochester, for his work on pigment metabolism and regeneration of metabolism.

Edward N. Gibbs Memorial Prize.—It is announced by the New York Academy of Medicine, 2 East 103d Street, New York City, that a sum of approximately $1,000 is available under the Edward N. Gibbs Memorial Prize toward original research in diseases of the kidney during 1938.

Candidates must be physicians who have been graduated at least three years and residents of the United States. They shall submit "evidence of research already performed and of facilities to prosecute research upon the causation, pathology and new methods of treatment of diseases of the kidney."

The award may be continued through not more than three years to any one person.

Applications with the required evidence should be addressed to the New York Academy of Medicine prior to June 1.

Society News.—The next meeting of the Federation of American Societies of Experimental Biology will be held in Toronto in April 1939.

The International Association of the History of Medicine will hold its eleventh congress in Yugoslavia, Sept. 2-11, 1938. Sessions will be held successively in Zagreb, Belgrade, Sarajevo and Ragusa.

The American Public Health Association will hold its sixty-seventh annual meeting in Kansas City, Mo., Oct. 25-28, 1938.

The Third International Cancer Congress under the auspices of the International Union against Cancer will be held Sept. 11-16, 1939, at the Haddon Hall Hotel, Atlantic City, N. J. The president is Francis Carter Wood, New York City. Donald S. Childs, Syracuse, N. Y., is the secretary-treasurer, and A. L. Loomis Bell, Brooklyn, is in charge of transportation and exhibits. The proposed sections are as follows: general research; biophysics; genetics; general pathology of cancer; surgery of cancer; roentgenologic diagnosis of cancer; radiotherapy of cancer; statistics, and education. The membership fee will be $15. Inquiries should be addressed to the Institute of Cancer Research, 1145 Amsterdam Avenue, New York City.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Pathologic Anatomy

Myocardial Abscess with Perforation of the Heart. S. Weiss and R. W. Wilkins, Am. J. M. Sc. **194**:199, 1937.

Two cases of solitary myocardial abscess perforating the heart are described. In the first case the abscess, caused by Staphylococcus aureus, ruptured into the right ventricle as well as into the pericardial sac. In the second case the abscess, caused by Pneumococcus, was localized mainly in the fat tissue at the junction of the auricle and ventricle and was formed as a direct extension of a small mural pneumococcic vegetation. This abscess ruptured into the pericardial sac. In both cases the clinical course did not suggest sepsis and the cardiac perforation resulting in fatal circulatory collapse was unexpected. While abscess of the myocardium is not an unusual occurrence in cases of sepsis, perforation of the heart by such an abscess is rare. Seven instances, collected from the literature, are cited. As judged from the 9 cases now available, myocardial abscess is silent until its rupture causes sudden pain low in the thorax, followed by circulatory collapse. In 2 of the 9 cases a diastolic murmur was present. From Authors' Summary.

Post-Traumatic Internal Hydrocephalus. A. R. Moritz and W. B. Wartman, Am. J. M. Sc. **195**:65, 1938.

In 4 cases of injury to the head followed by chronic internal hydrocephalus each patient so far as known was normal before the injury. In every instance the injury caused temporary loss of consciousness. Indications of internal hydrocephalus developed from two weeks to eight years after the injury and progressed with varying rapidity to the time of death. Postmortem examination disclosed dilatation of all the ventricles as a result of chronic obstructive leptomeningitis in the region of the roof of the fourth ventricle. The leptomeningeal adhesions contained hemosiderin, and probably they resulted from the organization of a subdural hematoma caused by the injury. From Authors' Summary.

The Pathology and Pathogenesis of Clinical Acute Nephritis. E. T. Bell, Am. J. Path. **13**:497, 1937.

Bell has classified 110 cases of acute nephritis in accordance with the structural changes in the kidneys. There were 31 cases of uncomplicated acute glomerulonephritis and 20 cases of nephritis associated with another disease. Obstruction of the glomerular circulation is usually due to endothelial proliferation, but in a few instances it is due partly or largely to the presence of epithelial crescents, intracapillary thromboses, thromboses in arterioles or polymorphonuclear leukocytes. In the normal state of the glomerulus and in subclinical glomerulonephritis it may be seen that all the capillaries of the lobules are completely invested with a basement membrane, but in clinical glomerulonephritis the capillaries within the lobule become fused and the inner basement membrane of each splits to form the characteristic intracapillary fibers. The lesions are all intracapillary; the appearance of an "intercapillary" lesion is due to the persistence of a portion of a capillary lumen in a peripheral part of the affected lobule. In 5 cases uremia was due to numerous massive lesions of the embolic type, in the absence of endocarditis.

In 11 cases the outstanding symptoms were septicemia, hematuria and uremia. This is called the hemorrhagic type of glomerulonephritis. The blood escapes through ruptured glomerular capillaries, and uremia is due to obstruction of the tubules by masses of red blood cells or of hemoglobin. Albuminuria hematuria and edema of renal origin are evidences of glomerular injury; tubular disease is evidenced by oliguria and anuria. In rare instances acute uremia is due to multiple thrombosis of small renal arteries. The most frequent form of tubular nephritis is that associated with mercuric chloride poisoning, but in occasional instances tubular disease is due to other causes. The acute uremia following transfusion of incompatible blood is due chiefly to obstruction of the collecting tubules by casts of hemoglobin. There is a group of cases in which uremia seems to be due partly to extrarenal conditions and partly to distention of the convoluted tubules, with minor degenerative changes in their lining cells. In uremia due to purely extrarenal causes the kidneys are normal, and the azotemia is due chiefly to dehydration and to increased destruction of protein. Decrease of blood chloride is apparently not a cause of azotemia. The azotemia of diabetic coma is in some instances due in part to tubular injury. FROM AUTHOR'S SUMMARY.

PARATHYROID HYPERPLASIA IN CHRONIC RENAL INSUFFICIENCY. B. CASTLEMAN and T. B. MALLORY, Am. J. Path. **13**:553, 1937.

Another case of "primary" hyperparathyroidism characterized by diffuse hyperplasia of the parathyroid glands of the water-clear type is reported. The histologic observations in this case have been used to emphasize the contrasting character of the "secondary" hyperplasia, which is described in detail on the basis of twenty-nine cases of chronic renal insufficiency of varying grades. Whereas in primary hyperplasia a uniform direction of differentiation of all cells to the large water-clear type is always present, in secondary hyperplasia such uniformity is lacking. In this condition the glands are composed almost entirely of chief cells of normal size, although a few water-clear cells are occasionally present. The oxyphil cells are always greatly increased in number. The glands show grossly varying degrees of enlargement, and even when the enlargement is limited to a single gland, microscopic examination has not failed to show hyperplasia in the other glands as well. The criteria for the diagnosis of secondary hyperplasia are described. A comparison of cases of chronic renal insufficiency with and without lesions of bones showed quantitative but not qualitative differences in the parathyroid glands, and the development of osteitis fibrosa is felt to be directly dependent on the duration of renal insufficiency. That these changes are in no way specific for renal insufficiency is shown by the fact that no qualitative differences could be recognized between the milder grades of secondary hyperplasia in cases of nephritis and those occasionally seen in persons without renal insufficiency but with a variety of associated lesions varying from metastatic carcinomatosis involving bone to basophilism of the pituitary gland. FROM AUTHORS' SUMMARY.

ARTERIOLAR SCLEROSIS IN HYPERTENSIVE AND NONHYPERTENSIVE PERSONS. A. R. MORITZ and M. R. OLDT, Am. J. Path. **13**:679, 1937.

The three principal histologic types of chronic arteriolar disease include (1) intimal hyalinization, (2) medial hypertrophy and degeneration and (3) intimal proliferation. All three types were found separately and in combination, in nonhypertensive as well as in hypertensive persons. Although no direct information was available as to the etiologic background of the three types, there was indirect evidence in support of the following views: 1. Intimal hyalinization is the arteriolar counterpart of simple arteriosclerosis, is essentially a degenerative change and becomes more widespread and severe with advancing age. 2. Medial hypertrophy and degeneration are changes resembling those following distention of any hollow muscular structure, and although medial degeneration may be

primary in some instances, yet it is probably secondary to stretching and has been seen with greater frequency and severity in hypertensive than in nonhypertensive persons. 3. Endothelial hyperplasia with increase in elastic tissue and secondary degenerative changes was seen commonly in association with inflammation, when it was properly called endarteritis obliterans, commonly as an adaptive involutional change in vessels the capillary beds of which had been reduced by parenchymal atrophy and commonly in cases of hypertension, in which it may have represented primary vascular inflammation or accelerated arteriolar sclerosis. It is concluded that the only significant site of arteriolar sclerosis so far as the causation of hypertension is concerned is the kidney.

FROM AUTHORS' SUMMARY.

THE ROLE OF RHEUMATIC FEVER IN THE IMPLANTATION OF BACTERIAL ENDOCARDITIS. L. GROSS and B. M. FRIED, Am. J. Path. **13**:769, 1937.

There have been described in this report macroscopic and microscopic observations in the hearts in 42 cases of subacute bacterial endocarditis and 28 cases of acute bacterial endocarditis. It is shown that while there is no sharp line of distinction between these conditions, a variety of lesions being common to both, certain clinical, bacteriologic, anatomic and histologic features are of considerable aid in classifying cases of bacterial endocarditis into these categories. An important, differentiating histologic feature is the spongy lesion that occurs in its typical form, perhaps exclusively, in subacute bacterial endocarditis. Through the presence of certain histologic stigmas of rheumatic fever it is possible to recognize bacterial endocarditis that is superimposed on previous rheumatic damage. In 75 per cent of these cases the heart had been the seat of a previous rheumatic process. In a discussion of the mechanisms involved in the implantation of bacterial endocarditis, reasons are given for believing that activity of a rheumatic infection is not a necessary precursor of bacterial endocarditis. Aschoff bodies were encountered in approximately 30 per cent of the cases in which acute and subacute bacterial endocarditis were superimposed on a rheumatic damage, and evidence is presented supporting the view that in some of these cases the rheumatic process was thrown into activity by the superimposed bacterial infection rather than that the activity of the rheumatic process predisposed to the bacterial endocarditis. Certain mechanisms are suggested as the means by which the endocardial structures are predisposed to bacterial implantation. These mechanisms include eosinophilic necrosis of the closure line of the valve, and thrombotic, proliferative and necrotic changes at this site. Some of these alterations are brought about by the hemodynamics present in hearts showing congenital and acquired defects. Others are probably due to inflammatory, toxic or degenerative processes. The endocardial alterations, together with intracardial tension, seem to predispose the endocardial structures of the heart to bacterial implantation by providing suitable means for anchoring transient bacterial invaders. Some of these mechanisms are present in nonrheumatic valves, but less frequently than in rheumatic valves. It does not appear that the vascularization occurring in rheumatic valves plays an appreciable role in the implantation of bacteria. FROM AUTHORS' SUMMARY.

HISTOLOGIC BASIS FOR ROENTGEN DIAGNOSIS OF PULMONARY MILIARY TUBERCULOSIS. P. E. STEINER, Am. Rev. Tuberc. **36**:692, 1937.

A comparison of the roentgenograms of patients dead of miliary tuberculosis with the number, size and structure of the tubercles in the lungs revealed that the latter factor shows the best correlation with the appearances in the roentgenogram. In all cases in which the tubercles were epithelioid, the roentgenograms made before death gave no evidence of tuberculous change whereas in cases in which the tubercles contained caseous material or collagen or both the roentgenograms usually showed evidence of this change. The chemical explanation for this fact

is not known. None of the tubercles contained visible calcium as studied with routine stains, and microincineration of tubercles of the various histologic types revealed no significant quantitative difference in ash. By using softer roentgen rays or by selective filtration of the rays of certain wavelengths, epithelioid tubercles might be made to produce positive indications in the roentgenogram.

H. J. CORPER.

STRUCTURE AND FUNCTION OF THE VERMIFORM APPENDIX IN THE GENESIS OF APPENDICITIS. O. H. WANGENSTEEN and others, Ann. Surg. **106**:910, 1937.

The vermiform appendix in man serves no known useful function. It apparently has a function, however, namely, the secretion of fluid. This is why the appendix is treacherous when obstructed. What the nature of the fluid secreted by the appendix is has not been determined. How the lumen of the appendix may become obstructed in the absence of an evident cause remains to be demonstrated.

The minute anatomy of the appendix as it bears on the problem of acute appendicitis has been broadly studied. Its resistance to any outflow from its lumen is in all likelihood a matter of its small luminal capacity and strong sphincter-like circular muscle. No evidence of a true cecoappendical sphincter has been found.

The effects of obstruction of the cecal appendage have been studied in many animals; only the appendix of the rabbit has so far been found to possess a secretory function similar to that evidenced in the vermiform appendix of man.

FROM AUTHORS' SUMMARY.

VASCULAR OCCLUSION IN MULTIPLE SCLEROSIS AND "ENCEPHALOMYELITIS." T. J. PUTNAM, Arch. Neurol. & Psychiat. **37**:1298, 1937.

According to Putnam, encephalomyelitis is merely a more acute stage of multiple sclerosis and, like the latter, is due to local obstruction of veins (thrombosis). The primary lesion is in the blood; the degeneration of the myelin and the glial reactions are secondary. Venous obstruction, experimental or observed in pathologic human material, produces demyelination more readily than destruction of the gray matter. Thrombi were observed by Putnam in 9 of 17 cases of multiple sclerosis and consisted of agglutinated platelets with or without proliferation of endothelial cells or degeneration of the vascular wall. More common was obstruction of the vessels by fibrous plugs. This was observed in 14 cases. The thrombi were usually in groups; inflammatory changes were slight, but other vascular phenomena—engorgement and slight hemorrhages—were frequent, especially in areas of more acute disturbance.

GEORGE B. HASSIN.

MARIE'S ATAXIA (OLIVOPONTOCEREBELLAR ATROPHY). GEORGE B. HASSIN, Arch. Neurol. & Psychiat. **37**:1371, 1937.

Marie's ataxia, which is the same as olivopontocerebellar atrophy, is rarer than Friedreich's ataxia and may give a picture of multiple sclerosis.

A man aged 52 complained of dizziness, tinnitus, unsteadiness of gait and disturbances of speech. He exhibited adiadokokinesis, ataxia, exaggerated reflexes and urinary disturbances, which were the cause of death. Microscopic observations revealed atrophy of the basal portion of the pons, of the pontile brachia (middle peduncles of the cerebellum), of the white substances of some lamellae of the cerebellum and of the olivocerebellar fibers, destruction of the ganglion cells of the pons and olives, with progressive reactive changes in the glia, a normal state of the pyramidal fibers and enormous cellularity of the cerebellar white substance. In short, the pathologic picture was that of olivopontocerebellar atrophy, which is usually familial and hereditary.

GEORGE B. HASSIN.

VASCULAR ARCHITECTURE OF THE LESIONS OF MULTIPLE SCLEROSIS. T. J. PUTNAM and A. ADLER, Arch. Neurol. & Psychiat. **38**:1, 1937.

Putnam and Adler studied the vascular structure of three typical plaques chosen from cases of multiple sclerosis. Reconstruction of the plaques as glass models convinced them that the plaques of multiple sclerosis possess a characteristic vascular architecture; that small plaques tend to surround engorged veins, which when thrombosed are associated with signs of fresh parenchymal degeneration in their vicinity, and that the histologic changes seen in multiple sclerosis are in general of vascular origin.

GEORGE B. HASSIN.

LISSAUER'S DEMENTIA PARALYTICA: A STUDY OF ITS PATHOGENESIS. N. MALAMUD, Arch. Neurol. & Psychiat. **38**:27, 1937.

A histologic study of 4 cases of dementia paralytica of Lissauer's type (alternating epileptiform and apoplectiform attacks with residual focal symptoms) showed prevalence of parenchymal changes (status spongiosus, laminar destruction of the cortex, proliferation of blood vessels and comparatively few inflammatory phenomena). Malamud accepts the theory that this condition has its origin in the action of a toxin. Toxins produce colloidal changes (synäresis) in the brain tissues which are responsible for the parenchymal degeneration, though there may be contributing factors (inflammatory, metabolic, vascular or other).

GEORGE B. HASSIN.

Immunology

INFLUENCE OF ALLERGY ON THE DEVELOPMENT OF EARLY TUBERCULOUS LESIONS. L. DIENES and T. B. MALLORY, Am. J. Path. **13**:897, 1937.

A group of experiments designed to clarify the correlation of the early tissue response to tuberculous infection with the development of allergy is described and illustrated. Guinea pigs with massive primary infection of the peritoneal cavity or testicles, in which reinfection lesions were produced on successive days, were simultaneously given skin tests for tuberculin sensitivity. After the lapse of varying periods of time the primary and reinfection lesions and the tuberculin reactions were compared histologically. Under the conditions employed and within the limits of error of the method, it was found that the first appearance of significant numbers of large mononuclear cells and the development of detectable sensitivity to tuberculin occurred simultaneously between the third and fourth days after the primary infection. The significance of these observations in relation to the authors' previously advanced hypothesis that bacterial allergy is responsible for the early granulomatous reaction of the host has been discussed.

FROM AUTHORS' SUMMARY.

IMMUNIZATION OF GUINEA PIGS WITH A MODIFIED STRAIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS. E. TRAUB, J. Exper. Med. **66**:317, 1937.

A strain of choriomeningitis virus which was highly virulent for guinea pigs, as isolated from a naturally infected white mouse, has been markedly attenuated for guinea pigs by serial intracerebral passage through white mice. The change of virulence occurred before the eighth serial passage. The modified virus as a rule produces fever in guinea pigs but no other symptom, and the infection is followed by solid immunity. Parallel passages of the same strain through guinea pigs maintained its high virulence for this species but reduced slightly its pathogenicity for mice. These observations indicate that the difference in virulence for guinea pigs noted before with different strains of choriomeningitis virus obtained from infected stock mice may be due not only to difference in the susceptibility

of the guinea pigs but also to variation in the virulence of the virus. A marked degree of resistance was demonstrable in several guinea pigs on the fourth, eighth and tenth days after injection of the modified virus, when antivirus could not yet be detected in the serum. Circulating antivirus appears, therefore, to play a secondary part in their immunity, which seems to be closely associated with the tissues, as in mice.

FROM AUTHOR'S SUMMARY.

SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS: ANAPHYLAXIS INDUCED BY PICRYL CHLORIDE AND 2,4-DINITROCHLOROBENZENE. K. LANDSTEINER and M. W. CHASE, J. Exper. Med. **66**:337, 1937.

It has been shown that by the cutaneous administration of simple chemical compounds in small quantities—2,4,6-trinitrochlorobenzene, the latter a typical incitant of contact dermatitis in man—it is possible to induce true anaphylactic sensitization in guinea pigs, demonstrable by an intravenous injection of protein conjugates and by the Dale technic, using isolated uterine horns. This furnishes strong evidence for the formation of antigenic conjugates following application of substances of simple chemical constitution. Since the anaphylactic state is induced by the same method of administration that gives rise to cutaneous sensitivity, the assumption appears justified, when one takes into account the chemical properties of the inciting substances, that the formation of conjugated antigens offers an explanation for the cutaneous effects also. In the experiments with picryl chloride, anaphylactic antibodies and occasionally precipitins have been demonstrated. The differences between the cutaneous and anaphylactic types of sensitivity are discussed.

FROM AUTHORS' SUMMARY.

THE COMPOSITION OF SPECIFIC PRECIPITATES IN THE REGION OF ANTIGEN EXCESS. S. MALKIEL and W. C. BOYD, J. Exper. Med. **66**:383, 1937.

Data are given, for seven different antiserums, on the composition of the specific precipitate as a function of the relative proportions of antiserum and antigen used. In the region of antigen excess, a linear relation is found between the ratio of antibody to antigen in the precipitate and the amount of antiserum used. The significance of these results, particularly in their bearing on theories of the precipitin reaction, is discussed.

FROM AUTHORS' SUMMARY.

TYPE-SPECIFIC PROTEINS OF ANTIPNEUMOCOCCUS SERUM. K. GOODNER and F. L. HORSFALL JR., J. Exper. Med. **66**:413, 425 and 437, 1937.

The ability to carry out with some measure of precision mouse protection tests for the estimation of the potency of antipneumococcus serum has made possible the correlation of the protective potency with the amount of specifically precipitable protein. With antipneumococcus rabbit serum these protective ratios are relatively constant and higher than those with immune horse serum. Type I antipneumococcus horse serums, on the other hand, show no such constancy but fall into two groups, and there is as yet no simple method for determining to which group a serum belongs.

In an effort to explain a certain lack of parallelism between the antibody content and the protective value of antipneumococcus horse serum, experiments have been carried out in which determined proportions of the specific antibody have been removed from the serum and the protective value of each residuum determined. This experimental procedure has revealed that the specific antibodies of immune serum do not possess uniform mouse protective potencies. The inference is that there may be several different substances in immune serum, each dominantly specific, yet exhibiting marked differences in avidity and therefore in protective value.

Immunologic and immunochemical fractionation of type I antipneumococcus horse and rabbit serums have demonstrated several forms of immune protein, each fraction behaving as a type-specific antibody, but differing from the others in chemical and immunologic properties. FROM AUTHORS' SUMMARIES.

STUDIES ON IMMUNITY IN A TYPE OF HUMAN ALLERGY (HAY FEVER). R. A. COOKE, M. LOVELESS and A. STULL, J. Exper. Med. **66**:689, 1937.

Large injections of ragweed pollen extract into normal nonsensitive volunteers did not produce sensitization to ragweed. Group 1, volunteers in whose skin many reactions were induced by injections of ragweed extract mixed with ragweed sensitive serum, failed to show any serologic changes. The theory that the immune substance found in the serum of treated ragweed-sensitive patients is due to the reaction or to some substance created by it and not to the ragweed per se was not upheld. On the contrary in group 2, volunteers who received larger amounts of ragweed but no sensitive serum, serologic changes were induced resembling those previously observed to occur in ragweed-sensitive patients after treatment. They were demonstrable by an inhibition of the immediate reaction and by interference with the neutralization of sensitive serum by its antigen. These serologic changes are therefore independent of the specific reaction characteristic of this type of allergy. The inhibiting factor was found to be related to the pseudoglobulin fraction of the serum and was shown to be specific.

FROM AUTHORS' SUMMARY.

PNEUMOCOCCIC TOXINS AND ANTITOXINS. A. F. COCA and others, J. Immunol. **32**:271, 1937.

About 20 per cent of the usual rabbits and almost all so-called Havana red rabbits reacted positively to human skin test doses of the toxin of Pneumococcus type III. Intraperitoneal injections of the toxin in doses varying from 3,000 to 30,000 skin test doses caused marked elevations of temperature. Rabbits inoculated repeatedly with the toxin produced an immune serum capable of neutralizing the toxin specifically. The neutralization was judged by the failure of the temperature to rise after the injection of the neutralized toxin as it did in nonprotected animals. A toxin was produced which was highly toxic for mice. A comparison on mice showed this toxin to be about one-half as potent as the strongest available scarlatinal toxin.

I. DAVIDSOHN.

DIFFERENCES IN THE ACTIVITY OF SERUM COMPLEMENT FROM VARIOUS SPECIES. E. W. SHRIGLEY and M. R. IRWIN, J. Immunol. **32**:281, 1937.

Normal serums of the ox, rabbit and sheep were found bactericidal for Brucella suis, while the serum of the guinea pig was not. Inactivated serums could be reactivated by fresh serums of other animals. To remove the normal antibodies, the serums were absorbed with heat-killed and washed suspensions of Brucella suis. The complementing ability differed greatly. Guinea pig serum failed to reactivate the inactivated serums of the ox, rabbit and sheep. Rabbit complement reactivated only rabbit serum. Ox complement reactivated the serums of the rabbit and ox and partly that of the sheep. Sheep complement had the widest range of reactivation. The results indicate differences in the complementing properties of different serums with regard to normal bactericidal serums against Brucella suis.

I. DAVIDSOHN.

HEREDITY OF AGGLUTINOGENS M AND N AMONG PUEBLO AND BLACKFEET INDIANS. F. W. ALLEN and D. LARSEN, J. Immunol. **32**:301, 1937.

The hypothesis of Landsteiner and Levine, according to which the factors M and N are inherited by means of a single pair of allelomorphic genes, was

tested on 140 Pueblo Indians. M+ N+ occurred in 32.86 per cent, M+ N— in 59.29 per cent and M— N+ in 7.85 per cent. The application of the mathematical formula of Wiener and Vaisberg brought forward results favoring the hypothesis of Landsteiner and Levine. The formula was also applied to the data for Blackfeet Indians that were published by Matson and his associates. The results were identical.

I. DAVIDSOHN.

BLOOD GROUPING TESTS ON THREE HUNDRED MUMMIES. W. C. BOYD and L. G. BOYD, J. Immunol. **32**:307, 1937.

Specimens from 122 Egyptian mummies and from 205 American mummies were tested for the presence of blood group factors A and B by means of absorption of isoagglutinins from human serums and of immune agglutinins from rabbit serums. The absorptive ability was weak, but the presence of A or B or of AB in some of the mummies seems established, even in mummies more than five thousand years old. The great frequency of AB was striking and difficult to explain. A large percentage of O was found (93 among 122 Egyptian mummies and 183 among 205 American), but it remains undetermined whether these were true O groups or members of the other blood groups from which factors (A or B) had been lost in the course of time. Precipitin tests with the serum of a rabbit immunized against human red cells were tried, but the results were not convincing.

I. DAVIDSOHN.

ACTIVE IMMUNIZATION AGAINST EPIDEMIC POLIOMYELITIS WITH RICINOLEATED VACCINE. J. A. KOLMER, J. Immunol. **32**:341, 1937.

From five to ten subcutaneous injections of from 0.05 to 1 cc. of ricinoleated vaccine protected 75 per cent of rhesus monkeys against intracerebral inoculations of virus. Larger doses of from 0.5 to 1 cc. protected from 93 to 100 per cent of 21 animals. Of 7 monkeys given subcutaneous injections, 5 were protected against intranasal instillations of the virus. Of 43 animals given intracutaneous inoculations, 77 per cent were protected, and such injections when small doses were used proved more efficient than subcutaneous injections. Kolmer emphasizes the importance of technical safegards for the proper evaluation of experimental findings; for instance, it must be established that the vaccine contains living virus, and the vaccine must be finely divided cord substance (Berkefeld filtrates were not satisfactory). In 3 of 183 subcutaneously vaccinated animals paralysis developed during immunization, but it did not in any of the 59 monkeys in which the virus had been inoculated intradermally. The presence of neutralizing antibody in 18 immunized monkeys protected them against intracerebral inoculation of the virus, but after an interval of eighteen days a larger intracerebral test inoculum caused paralysis of 3 animals. Kolmer concludes: "We believe that the antibody engendered by ricinoleated vaccine plays some role in the immunity of monkeys, but the immunity is relative and probably requires large amounts of antibody for the protection of the nervous system following intracerebral and intranasal inoculations with virus."

I. DAVIDSOHN.

EFFECT OF ACTIVE IMMUNIZATION ON THE LIPID CONTENT AND PHAGOCYTIC ACTIVITY OF LEUKOCYTES OF RABBITS. E. M. BOYD, J. H. ORR and G. B. REED, J. Immunol. **32**:415, 1937.

Boyd had demonstrated variations in the phospholipid content of human leukocytes and suggested that the greater the phospholipid content of the leukocytes in the blood of a patient with infection the greater are the chances of recovery. To study the effect of immunization on the phospholipids of leukocytes, rabbits were inoculated repeatedly with Streptococcus viridans. The results showed no rise of the lipids, indicating that the increases observed in the course of certain human infections must be associated with other manifestations of the pathologic process than with the rising immunity.

I. DAVIDSOHN.

TYPE-SPECIFIC CARBOHYDRATES OF GONOCOCCI. W. A. CASPER, J. Immunol. **32**: 421, 1937.

The technic of isolating a carbohydrate fraction from gonococci was described. It permits separation of the gonococci into two serologically distinct types. The differentiation is particularly clearcut in skin reactions to injections of the type-specific carbohydrates. It is essential that fresh strains be used for the preparation of the carbohydrate fraction. Old degenerated strains, also those isolated from persons with chronic gonorrhea, proved unsatisfactory, mainly on account of the absence of type specificity. Overlapping reactions were due to the presence of species-specific proteins.

I. DAVIDSOHN.

PRESENCE IN THE SAME NORMAL RABBIT SERUM OF ANTI-N AND ANTI-Q AGGLUTININS. S. ISEKI and T. FUKAO, Hanzaigaku-Zasshi **10**:657, 1936.

The particular normal rabbit serum tested agglutinated human red blood cells of all groups and types in approximately equal dilutions in direct tests. When proper preliminary absorption tests were done, however, the serum was found to contain a weak anti-Q and a moderately potent anti-N agglutinin.

A. S. WIENER.

RELATION BETWEEN BLOOD TYPES E AND e, BLOOD GROUPS, M-N TYPES AND THE SUBGROUPS. S. SUGISHITA, Juzenkai-Zasshi **40**:2341, 1935.

No relation was found between the so-called properties E and e and any of the previously described agglutinogens in human blood.

A. S. WIENER.

HEREDITY OF THE BLOOD TYPES E AND e. S. SUGISHITA, Juzenkai-Zasshi **40**:3367, 1935.

Property E was found to be dominant over property e. In particular, in 10 families in which both parents belonged to type e, all 21 children were found to belong to type e.

A. S. WIENER.

Tumors

ASTROCYTOMA. B. J. ALPERS and S. N. ROWE, Am. J. Cancer **30**:1, 1937.

The practical advantages of a clinicopathologic classification of a group of varying tumors of the brain are obvious. With the accumulation of pathologic data and the more careful delineation of clinical pictures, more accurate preoperative diagnoses may be achieved, and the operative attack on the lesion may be planned and executed more intelligently. After histologic examination of the tumor, either through immediate biopsy or through postoperative examination, such vital questions as the radicalism of the removal, the advisability of irradiation, the probabilities of recurrence and the ultimate prognosis may be answered with reasonable accuracy. Briefly the classification of astrocytoma which Alpers and Rowe have proposed is as follows:

I. Fibrillary
  1. Solid
    (*a*) Piloid
    (*b*) Diffuse
  2. Cystic

II. Giant cell

III. Cellular

The division of astrocytoma into these three groups serves a definite practical purpose. It indicates, first of all, the great preponderance of the fibrillary group

and makes possible in the majority of instances a safe prediction of the course which the tumor will take. In general, tumors of the fibrillary groups grow slowly and have a correspondingly slow clinical course. The piloid subgroup of the solid fibrillary group, while benign histologically, seems to be less benign in clinical features. Tumors of the giant cell and cellular groups grow more rapidly and correspondingly follow a more rapid clinical course than those of the fibrillary group. The large main group of astrocytoma, the fibrillary group, is in good agreement with the investigations of others (Cushing and Bailey; Hortega; Roussy and Oberling). Penfield classified his fibrillary group as the piloid group. It has been the experience of Alpers and Rowe, however, that the piloid type of tumor is better regarded as a subdivision of the larger fibrillary group. Only 26 of the 112 fibrillary tumors collected by Alpers and Rowe could be regarded as piloid, with the features described by Penfield: (*a*) long fibrils arranged in bundles and (*b*) a few elongated cells having some but not all the features of classic astrocytes. Since the type cell in these tumors is the astrocyte, and since, also, the outstanding characteristic of the group is the presence of neuroglia fibrils, it has seemed to the authors that these tumors are best regarded as representing a fibrillary type of astrocytoma with special histologic characteristics but not with pathognomonic clinical features. Most of the tumors in the fibrillary group are characterized by the diffuse interlacing neuroglial carpet, which simulates the neuroglial arrangement in the normal white matter. The reason for the difference in appearance of these slowly growing tumors must remain at present a matter of speculation: Whether it is due to a difference in the inherent properties of the astrocytes or to the effects of exogenous stimuli carried to the cells by the blood is not clear. Nor is it clear why the number of fibrils within the tumors of the fibrillary group varies so greatly; why, for example, there are many fibrils and few cells in some tumors and many cells but few fibrils in others. In the former group the number of fibrils is so great that, while it is conceivable that they have been deposited by the few cells which lie among them, it is hard to dismiss the possibility of their formation from some other source. A similar preponderance of fibrils over cells is seen in some cases of fibroblastoma and in certain cases of gliosis in the brain. Granting that the preponderance of fibrils is due entirely to their formation by the cells, it is still not evident what the stimulus is for their being so heavily deposited in some tumors as compared with others, nor why some of these tumors develop as the diffuse type and others as the piloid type, all of them being the product presumably of the same cell, the fibrillary astrocyte. No attempt has been made to divide astrocytoma into fibrillary and protoplasmic types. In all the fibrillary tumors it is possible to find protoplasmic types of astrocytes as well as immature forms. It may be argued that the cellular form of astrocytoma which the authors have described is in reality the protoplasmic type, but even in this form there is some fibrillar formation. Since it is not clear whether the lack of fibrils is due to the presence of protoplasmic astrocytes or to the presence of immature cells, it has been deemed best to refer to these tumors as cellular astrocytoma. Similarly there is little doubt that the giant cell growths constitute a group in themselves. In appearance and structure they are quite different from the other groups.

FROM AUTHORS' SUMMARY.

MELANOBLASTOSIS AND MELANOBLASTOMA: AN ANATOMIC STUDY. M. M. KESSLER, Am. J. Cancer **30**:19, 1937.

There are benign and malignant conditions involving melanoblastic cells. The benign conditions are congenital allotments of cells with melanoblastic functions to certain superficial tissues, the skin and its derivatives. This may be considered a normal distribution, as in the skin of the Negro, or heterotopic, as in the case of pigment cells found in the cerebrospinal meninges. The malignant conditions arise from sarcomatous or carcinomatous changes in these cells wherever they are

situated. The melanoblastic cells arise from both ectodermal and mesodermal tissues. The following classification of melanotic conditions is suggested:

I. Non-neoplastic

1. Mesodermal melanoblastosis: A normal, though heterotopic, incidence of mesoblastic melanoblasts in sufficient numbers to produce noticeable discoloration of the structure involved. Example: Pigmented cells in the meninges.

2. Epithelial melanoblastosis: A normal incidence of epithelial melanoblasts in sufficient numbers to be noticeable. Example: Skin of the Negro; epithelial inclusions in the brain.

II. Neoplastic

1. Benign

(*a*) Mesodermal melanoblastoma: A slowly proliferating (or quiescent) discrete accumulation of mesodermal melanoblasts in any of the sites wherein such cells are normally found. Example: The blue nevus of Jadassohn; also, an accumulation of such cells within the brain or meninges in circumscribed nodular collections.

(*b*) Epithelial melanoblastoma: A slowly growing or quiescent discrete proliferation of epithelial melanoblasts occurring in any of the situations in which such melanoblasts are normally found. This group name may be modified by the term "nevoid" whenever the specialized nevus cell is met, or by "basal cell" if the growth chiefly involves this cell. Example: The ordinary brown fleshy mole encountered on the face.

2. Malignant

(*a*) Melanosarcoma: A rapidly proliferating, usually widely metastasizing melanin-forming tumor which answers the pathologic criteria of a malignant tumor arising in connective tissue. Example: A pigmented tumor arising in the choroid of the eye.

(*b*) Melanocarcinoma: A rapidly proliferating, usually widely metastasizing melanin-forming tumor which answers the pathologic criteria of a tumor arising in epithelial tissue.

FROM AUTHOR'S SUMMARY.

INTRINSIC FACTORS IN THE ETIOLOGY OF NEOPLASMS. C. V. WELLER, Am. J. Cancer **30**:39, 1937.

Since cell division and tissue growth are intrinsic attributes of every metazoan organism, every organism possesses the basic intrinsic factors essential for neoplastic growth. Neoplasia, like all other disease processes, results from the combined action of intrinsic factors. If the extrinsic factor is sufficiently potent, it is conceivable that neoplasia may be induced in any organism. In addition to this universal intrinsic attribute, the actual occurrence, type and site of a neoplasm are determined in part by specific intrinsic factors, but to varying degrees and in different ways for different new growths. There is no gene for cancer as a whole or for "noncancer." The significance of intrinsic factors, the part played by genes and by extrachromosomal factors, and the mendelian implications, if any, must be worked out separately for each kind of neoplasm. In certain instances a neoplasm develops on morphologic or functional abnormalities which are themselves intrinsic and inheritable. Some of these are dominant and some recessive in the mendelian sense, and the resulting neoplasm tends to approximate the hereditary pattern of the lesion on which they develop.

FROM AUTHOR'S SUMMARY.

SEX HORMONES AND THEIR RELATION TO TUMORS. L. LOEB and others, Am. J. Cancer **30**:47, 1937.

It must be left to further investigations to determine whether an extrinsic virus is active also in cancers due to gonadotropic substances and whether or not two entirely different types of tumors exist, one dependent during its whole course on the presence of a virus, and the other due to intracellular changes which are self-perpetuating. FROM AUTHORS' CONCLUSIONS.

A TRANSMISSIBLE LEUKEMIA IN MICE WITH ATYPICAL CELLS. W. A. BARNES and J. FURTH, Am. J. Cancer **30**:75, 1937.

A transmissible strain of leukemia in mice is described, the malignant cells of which are not typically lymphoid, myeloid or monocytic. The cells are not phagocytic but form giant cells similar to megakaryocytes and the giant cells of Hodgkin's disease. Transmission of leukemia is accomplished with material containing living cells, but fails with cell-free material. The length of life after inoculation is in inverse relation to the number of leukemic cells injected. The disease is readily transmitted to mice of the stock in which it originated. Unrelated mice may be rendered susceptible to the disease by massive repeated doses of roentgen rays but not by a single irradiation. Leukemic tumor tissue slowly frozen to —70 C. transmits the disease, while that rapidly frozen to —30 C. is entirely inactive. Slowly frozen leukemic tissue kept at —70 C. during thirty-two days produces the disease as readily as that kept at this temperature for thirty minutes. These studies indicate that successful inoculations with frozen material are due to a few cells which have escaped destruction. FROM AUTHORS' SUMMARY.

BOECK'S SARCOID (SARCOIDOSIS). W. T. LONGCOPE and J. W. PIERSON, Bull. Johns Hopkins Hosp. **60**:223, 1937.

Eight cases of generalized Boeck's sarcoid are described, and reference is made to an enlargement of the lymph nodes that occurred in ten additional patients. The disease pursued the course of a chronic generalized infection. It produced few constitutional symptoms but resulted in remarkable pathologic changes in many structures of the body. Although the condition has been most often described as a disease of the skin, characteristic changes in the skin were observed only in one of the eight cases. Emphasis is placed on the generalized nature of the process and on the frequency with which an enlargement of particular groups of lymph nodes occurs in this disease. Those most commonly involved are the preauricular and postauricular, the submaxillary and submental, the epitrochlear and the femoral or inguinal nodes. One or more of these groups was enlarged in all cases. Involvement of the roots of the lungs or of the mediastinum is also common and was observed in seven of the cases. The changes produced somewhat characteristic shadows in the roentgenogram. The alterations in the small bones of the hands, and feet, usually affecting the phalanges, seem to be specific for the disease. They produce perfectly typical shadows in the roentgenogram. Disease of the bones occurred in three of the patients, and in one they were unusually widespread. In two patients the spleen, and in two the liver, was enlarged. The lacrimal glands were swollen in two patients; the parotid gland was involved in one. Bilateral iridocyclitis occurred in one patient. Reference is made to the resemblance of some of the clinical features of uveoparotid fever to Boeck's sarcoid, and attention is drawn to the close similarity of the pathologic lesions in the two syndromes. The pathologic picture of Boeck's sarcoid reproduces almost exactly that of the miliary epithelioid tubercle without a surrounding inflammatory zone. Large pale central giant cells are sometimes seen, but areas of necrosis are exceptionally rare. The etiology of Boeck's sarcoid remains as yet undetermined. The disease is considered by many to be a peculiar form of tuberculosis. The tuberculin reaction is positive in only a small proportion of the cases. It was mildly positive in one of seven cases. In one instance a tuberculin reaction was not obtained. In the cases

of this series in which cultures were made from the lymph nodes, and in which guinea pigs, rabbits and pigeons were inoculated with material from the lymph nodes and from nasal scrapings, the results were all negative. Up to the present time the efforts of most of the investigators who have sought to prove that the disease is tuberculosis have failed, and one must conclude that the etiology of the disease remains unknown.

FROM AUTHORS' SUMMARY.

PRODUCTON OF DIBENZANTHRACENE TUMORS IN MICE. H. B. ANDERVONT and E. LORENZ, Pub. Health Rep. **52**:637, 1937.

Dog serum and horse serum dispersions of 1,2,5,6-dibenzanthracene were injected intravenously or subcutaneously, and lard solutions of the same compound were injected subcutaneously, into pure strain mice. The serum dispersions injected intravenously induced tumors in the lungs and liver, and those injected subcutaneously produced tumors at the sites of injection as well as in the lungs and liver. The lard dispersions injected subcutaneously evoked tumors at the sites of injection and also produced tumors in the lungs and liver.

The appearance of tumors in the lungs and liver in mice given the subcutaneous injections is evidence that 1,2,5,6-dibenzanthracene is capable of producing tumors in tissues which are distinct from the sites of injection.

FROM AUTHORS' SUMMARY.

INCIDENCE OF SPONTANEOUS TUMORS IN AN INBRED MOUSE STRAIN. H. B. ANDERVONT, Pub. Health Rep. **52**:772, 1937.

A record of sixteen generations of breeding females of mouse strain C_3H is submitted in this report. In these generations 1,300 mice have been or are under observation for the development of spontaneous carcinoma of the mammary gland. A high percentage of the mice in every generation have presented tumors, the incidence of such growths in the various generations ranging from 66.1 to 88.2 per cent.

By selecting mice of one particular line, the average age at which tumors appeared has been lowered from 14 to 15 months in the earlier generations to 8 to 10 months in the recent generations.

FROM AUTHOR'S SUMMARY.

TRANSFER OF TUMOR CELLS BY THE SURGICAL KNIFE. O. SAPHIR, Surg., Gynec. & Obst. **63**:775, 1936.

In biopsies of tumors the material on the knife blade invariably contained tumor cells.

MYXOMA AND THE SHOPE FIBROMA. E. W. HURST, Brit. J. Exper. Path. **18**:1, 1937.

Many of the observations of previous workers on the histologic aspects of myxoma have been confirmed. Certain additional appearances, notably those resulting from great hyperplasia of the vascular endothelium, have been described. The relative preponderance of destructive and proliferative lesions is no doubt partly dependent on the virulence of the infecting virus. In general, however, epithelial and some mesodermal structures, such as muscle, tend to degenerate and die when attacked by the virus, while other mesodermal structures, such as vascular and reticular endothelium, histiocytes and fibroblasts, tend to undergo considerable proliferation.

FROM AUTHOR'S SUMMARY.

EFFECT OF 1,2,5,6-DIBENZANTHRACENE OF SPONTANEOUS TUMORS OF MICE. F. C. PYBUS and E. W. MILLER, Brit. J. Exper. Path. **18**:126, 1937.

It is concluded that 1,2,5,6-dibenzanthracene has an inhibiting effect on the growth of spontaneous mammary tumors but that this effect is only temporary.

FROM AUTHORS' CONCLUSIONS.

PARTICULATE NATURE OF THE AGENTS OF AVIAN SARCOMA. C. R. AMIES, J. Path. & Bact. **44**:141, 1937.

A centrifugal force of from 20,000 to 30,000 times the force of gravity will deposit the whole of the tumor-exciting agent from highly active cell-free extracts of Rous sarcoma no. 1 and the Fujinami sarcoma. By employing the method of repeated fractional centrifugation it is possible to prepare highly active suspensions of these agents, which are apparently free from fowl protein. Microscopic examination of these purified suspensions reveals the presence of large numbers of particles of fairly uniform size and appearance which are below the limits of optical resolution. The available evidence suggests that each of these tumor agents exists in the form of these particles, which are of the nature of the elementary bodies of a virus. The purified suspensions are specifically agglutinated by the serums of fowls bearing the corresponding tumors. This reaction, however, cannot always be demonstrated. Some degree of cross-agglutination occurs between Rous and Fujinami suspensions and the corresponding antiserums. Agglutinins for both Rous and Fujinami suspensions can frequently be demonstrated in the serums of apparently normal adult fowls but not in those of normal young chickens. Serums which show a strongly positive agglutination reaction also contain neutralizing antibodies for the tumor agents. Purified suspensions which are apparently free from fowl protein are still neutralized by hyperimmune rabbit antifowl serums. The presence of complement does not appear to be essential for this reaction. From this finding it is inferred that the tumor agent contains an antigen which is normally present in fowl tissue. In view of the present uncertainty regarding the mode of action of neutralizing antibodies in virus infections, this conclusion may later require modification in the light of further knowledge.

FROM AUTHOR'S SUMMARY.

GENETIC AND ANTIGENIC BASIS OF TUMOR TRANSPLANTATION. P. A. GORER, J. Path. & Bact. **44**:691, 1937.

A sarcoma arising in a pure line of albino mice was found to be transferable to all other members of the line and to certain hybrids derived from it, but not to unrelated mice. Genetic investigation showed that two (possibly three) dominant genes must be present in the host or the tumor will regress. One of these genes appears to be identical with that determining the presence of an antigen detectable in the red blood cells of normal albino mice. The importance of this agglutinogen for the success of the transplantation is further demonstrated by the fact that isoagglutinins could be demonstrated in the serum of any mouse in which the tumor had recently regressed. These observations conform with the generally accepted view that individual differences of blood play an important role in the transplantation of tissues but that they are not the only genetic factors determining the success or failure of the transplantation.

A. S. WIENER.

IMMUNIZATION AGAINST MOUSE SARCOMA. A. BESSEMANS and L. ASAERT, Ann. Inst. Pasteur **58**:130, 1937.

Intradermal inoculation of a moderate dose of an emulsion of Ehrlich's sarcoma in mice was followed by death of half, quick recovery of a fourth, and no effect on the fourth of the animals. Subsequent inoculation with a dose normally fatal indicated a refractory state on the part of previously unaffected mice.

MAX MARSHALL.

BIOPSY. A. FEHR, Arch. f. klin. Chir. **187**:1, 1936.

In a series of 1,300 malignant neoplasms there were 288 in which biopsy was made, 248 of which proved to be malignant and 40 benign. The roentgenologist who irradiated accessible neoplasms of the skin and mucous membranes relied entirely on the biopsy for the determination of the nature of the growth and its

sensitiveness to roentgen rays. The biopsy gave the correct diagnosis in 87.8 per cent, the principal error being due to improper removal of the tissue to be examined. With proper precautions hemorrhage, infection and activation of the neoplastic growth become almost negligible. Biopsy had better not be made in cases of melanocarcinoma, because of the sensitiveness of this type of cancer to trauma.

MALIGNANT CRANIAL CHORDOMA. F. BOEMKE and W. JOEST, Virchows Arch. f. path. Anat. **297**:351, 1936.

This is a gross and microscopic study of two malignant chordomas of the base of the skull arising from the clivus. From the previous literature sixty-seven cases of cranial chordoma are tabulated as follows: chordoma of the clivus, twenty-four benign and twenty-four malignant; hypophysial, three; nasopharyngeal, ten; dental, one; other portions of the skull, four; exact origin not stated, one. The cells of the malignant chordoma correspond to those of the very early fetal notochord. The bone and cartilage found in the chordoma are derived secondarily from neighboring bone-forming and cartilage-forming tissue.

O. T. SCHULTZ.

MORPHOLOGY OF NEUROBLASTOMA OF THE ADRENAL MEDULLA. J. RINSCHEID, Virchows Arch. f. path. Anat. **297**:508, 1936.

Nonchromaffin tumors of the adrenal medulla with origin in the sympathetic system are classified as follows:

I. Neuroblastoma
   1. Sympathogonioma
   2. Sympathoblastoma

II. Ganglioneuroblastoma
   1. Differentiated: (*a*) ganglioneuroma immaturum; (*b*) ganglioneuroma imperfectum
   2. Partially differentiated: (*a*) neuroblastoma gangliocellulare; (*b*) proliferating ganglioneuroma

III. Ganglioneuroma simplex.

The morphologic study was directed chiefly toward the identification of neurofibrils by silver impregnation. Of four tumors studied, all in children, two were sympathogoniomas and two were sympathoblastomas. The sympathogoniomas consisted chiefly of very immature cells, sympathogonia, with a few sympathoblasts but with no fibril formation. Of the sympathoblastomas, one revealed intercellular fibril formation; the other, only intracellular fibrils. These differences in the sympathoblastomas Rinscheid interprets as evidence of differences in the degree of differentiation.

O. T. SCHULTZ

MALIGNANT PHEOCHROMOCYTOMA. G. KAULBACH, Zentralbl. f. allg. Path. u. path. Anat. **67**:49, 1937.

A pheochromocytoma the size of a child's head was found in the body of a woman 41 years old who died of cardiac decompensation. Tumor thrombi were found in the left adrenal vein and inferior vena cava, and tumor emboli throughout both lungs. An interesting complication in the clinical course was a series of adrenalogenic hypertensive crises.

GEORGE J. RUKSTINAT.

INCREASE OF GLYCOLYSIS FOLLOWING TARRING. W. BÜNGELER, Ztschr. f. Krebsforsch. **44**:141, 1936.

Büngeler, working in Fischer-Wasels' laboratory, was the first to discover a general shift of metabolism to that of the Warburg type in animals bearing native

tumors and in those treated with tar, indol and arsenic. Since this shift does not appear in animals in which tumors have been implanted, it would appear to be a primary alteration, which Büngeler interprets as evidence of the presence of a constitutional predisposition to cancer. H. E. Eggers.

Small Cells in Tumors. M. Borst, Ztschr. f. Krebsforsch. **44**:145, 1936.

Borst discusses colonies of small cells frequently seen at the margins of necrotic tumor tissue, especially in transplanted tumors and in the vicinity of hemorrhage. They seem to be atrophic tumor cells and in both location and character appear to lie between necrotic cells and those typical of the tumor. He is by no means certain that they are degenerating or dying cells; he suggests that they may be tumor cells which are utilizing necrotic material as a nutritive source and which are repressed in size by the barrier to expansion offered by the surrounding necrotic material. It is possible, he thinks, that they may be resting cell forms, capable of complete development in more favorable circumstances.

H. E. Eggers.

Synthetically Prepared Cancerogenic Substances and Their Relations to Physiologic Products. G. Domagk, Ztschr. f. Krebsforsch. **44**:160, 1936.

This summary includes a brief historical account and a detailed presentation of the study of the known cancerogenic agents and their relations to the gonadotropic substance and sterols. Domagk believes that the knowledge already obtained is sufficient for the initiation of prophylactic and therapeutic measures concerning diet and habit. H. E. Eggers.

Symmetrical Carcinoma. J. Körbler, Ztschr. f. Krebsforsch. **44**:448, 1937.

Körbler has found 7 cases of symmetrical primary carcinoma among the 2,370 cases of cancer encountered by him in the last five years. Even this number he regards as too great for such carcinomas to be the result of chance. Their explanation appears to lie in some early embryonic disturbance, before the establishment of a plane of symmetry, and so necessarily while the embryo is still composed of relatively few cells. The disturbance is evidently one that remains obscure until very much later in life and then usually becomes evident in response to some external irritation. Körbler surmises that such a disturbance may operate in the causation of cancer in general, since the symmetrical distribution would appear only when its focus lies across the future plane of symmetry. H. E. Eggers.

Primary Cancer of the Lung. E. Husted and G. Bülmann, Acta path. et microbiol. Scandinav. **14**:141, 1937.

The autopsies at the teaching hospital of the University of Copenhagen show a considerable increase in the number of cases of primary cancer of the lung. The present study deals with forty-one cases. No new light is thrown on the causation of the disease. A relation between the increasing frequency and tarring of roads could not be demonstrated.

Medicolegal Pathology

A New Antigen for the Serodiagnosis of Syphilis. A. Wadsworth and R. Brown, J. Immunol. **31**:155, 1936.

The infusion of beef heart with a 20 per cent solution of sodium chloride at 55 C. permitted later a more successful extraction with alcohol than was possible without the preliminary infusion. The maintenance of a proper p_H and the addition of 20 per cent dextrose to the diluting fluid were found important. A one tube precipitation reaction was used as a preliminary test for the elimination of negative serums. I. Davidsohn.

A Low Temperature Ball Mill for the Liberation of Labile Cellular Products. S. Mudd, C. H. Shaw, E. J. Czarnetzky and E. W. Flosdorf, J. Immunol. **32**:483, 1937.

Bacterial cells are dried in the lyophilic apparatus and then ground in a specially constructed low temperature ball mill. The cooling-down to —80 C. is accomplished by ethylene glycol monomethyl ether. The procedure is described in detail. In addition to bacteria, also tissues, wool, hair, pollen and rubber can be ground to a fine powder. The advantage of the new process is that the low temperature and absence of water prevent chemical reactions.

I. Davidsohn.

Migration of a Bullet from the Heart to the Popliteal Artery. Chavigny, Drout and Lamy, Ann. de méd. lég. **6**:698, 1937.

During a brawl a man was shot and died instantly. The bullet entered the left midaxillary line at the level of the fourth rib and passed through the left lung into the left auricle of the heart, where its course was lost. On roentgen examination, no bullet was found. The deep popliteal artery on each side was opened in order to explore the vessels where the roentgen representation stopped. The bullet was recovered at the level of the left deep popliteal artery, exactly at the point where it bifurcates into its terminal trunks.

Harvey S. Thatcher.

A New Method for Demonstrating Sperm for Forensic Purposes. K. Prägler, Deutsche Ztschr. f. d. ges. gerichtl. Med. **27**:143, 1936.

After the dry seminal stain is located on paper or cloth with the aid of a quartz lamp or by microchemical tests, it is extracted for twenty-four hours in 1.5 per cent antiformin. The material is then transferred to a glass slide, dried and fixed in the flame. It is stained by Baecchi's method; i. e., it is placed for from forty to fifty seconds in acid fuchsin, then for an equal length of time in 1 per cent hydrochloric acid and rinsed with absolute alcohol. By this method spermatozoa could be demonstrated in stains varying in duration from one to thirty-three years.

A. S. Wiener.

Histochemical Demonstration of Lead in Calcified Human Arteries. H. Kratsch, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:445, 1937.

In investigations of chronic lead poisoning and in experiments on the disposition of the poison in the body a parallelism has been demonstrated between the deposition and distribution of lead and calcium. Apparently enough lead is ingested with food to form demonstrable deposits in the bones and teeth. This affinity for lead in regions ordinarily rich in calcium obtains also in pathologic conditions. Decalcified plaques of sclerotic human aortas were treated with hydrogen sulfide to form the readily demonstrable lead sulfide. Sections examined under a microscope had a diffuse brown stain and, in addition, brown streaks and kernels near the junction of the intima and the media. In other regions of the intima and media there were round or oval places in each of which a brown border surrounded a necrotic cell mass. In dark field examinations, there was a luminescence in the particles which stretched beyond the borders, visible with ordinary light. Some of the blood vessels showed lead deposits close to their peripheries. The granules were arranged in irregular lines like strings of beads and simulated the haversian canals of bone. In this manner early metaplastic bone formation was demonstrated in sclerotic foci.

Guinea pigs fed lead carbonate in addition to their regular diet had no gross fatty or degenerated regions in their aortas after sixty days of such treatment. In dark field examinations, however, the intima-media zone and the vasa vasorum of the adventitia had lead deposits.

George J. Rukstinat.

POSTMORTEM NODULE FORMATION IN THE SKIN OF A "FLOATER." H. KLAUER and K. WALCHER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:464, 1937.

The skin of a "floater" immersed in sea water five weeks was green and the epidermis peeled off in large sheets. In the corium and dermis occurred numerous gritty nodules of pinpoint to pinhead size. These occurred with equal frequency near or at a distance from hair follicles. In paraffin or gelatin frozen sections there were bacterial masses on the cutis, and the nodules were composed of coarse crystalline needles. These proved to be calcium phosphate and apparently were formed in the following fashion: Calcium was derived from the sea water which soaked in through the skin. Phosphoric acid accumulated from the split products of protein and combined with the calcium. GEORGE J. RUKSTINAT.

Technical

STAIN FOR MYELIN SHEATHS IN TISSUES EMBEDDED IN PARAFFIN. G. S. MAHON, Arch. Neurol. & Psychiat. **38**:103, 1937.

Mahon describes a successful method of staining myelin fibers in paraffin or pyroxylin sections (from 20 to 40 microns thick). The reagents are: a 4 per cent aqueous solution of iron and ammonium sulfate, a saturated solution of lithium carbonate and a 10 per cent alcoholic solution of hematoxylin. Formaldehyde-fixed tissues are embedded in paraffin in the usual manner, and sections from 6 to 18 microns thick are mordanted (from fifteen to thirty minutes) in the solution of iron alum, rinsed two or three times in water and stained (for from thirty to sixty minutes) in lithium carbonate-hematoxylin; they are then washed in water (tap or distilled); they are transferred to a saturated solution of lithium carbonate and left in it for from fifteen to thirty minutes; they are then washed thoroughly, dehydrated and finally mounted in neutral xylene-balsam. The myelin sheaths are distinctly stained deep blue, the background remaining white. Frozen sections cannot be stained with this method. Nor is this technic good for the study of cortex. GEORGE B. HASSIN.

METHOD FOR RAPID IMPREGNATION OF MICROGLIA AND OLIGODENDROGLIA IN MATERIAL FIXED IN FORMALDEHYDE. L. S. KING and J. ANDERSON, Arch. Neurol. & Psychiat. **38**:362, 1937.

Frozen sections, from 20 to 25 microns thick, are received in tap water to which has been added 20 drops of ammonia per hundred cubic centimeters; they are then transferred first to a 5 per cent solution of ammonium bromide and heated in the paraffin oven at from 45 to 50 C. for from ten to fifteen minutes; they are then placed in Hortega's mixture (ammonia, pyridine and water, equal parts) for from two to three minutes and transferred from here to Hortega's strong solution of silver carbonate; after reduction in a solution of formaldehyde, the sections are washed, toned, fixed and mounted. As judged by three photomicrographs appended, the results are very satisfactory. GEORGE B. HASSIN.

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

C. PHILLIP MILLER, *President*

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PAUL R. CANNON, *Secretary*

EXPERIMENTAL SHOCK. FREDERICK M. ALLEN, Polyclinic Medical School and Hospital, New York.

Shock following constriction by a tourniquet has experimental advantages, producing characteristic symptoms (including reduction in blood and plasma volume) and death. The fatal limits have been standardized, especially in the rat, (1) *extensively* (amount of tissue ligated) and (2) *intensively* (length of time). The therapeutic results illustrate the nature of shock as follows: 1. Early amputation following ligation saves life. 2. Transfusion of blood or plasma succeeds in saving life within certain limits; similarly also, transfusion of blood derived from animals dying in shock, an observation indicating that there is in this blood no abundance of circulating toxin. 3. The amount of local transudation is governed largely by time; therefore it is least with severest, rapidly fatal shock; leakage of plasma elsewhere appears to be due to a constitutional cause. 4. With injury so extensive that blood or a solution of acacia fails to save life, abundant saline solution can still save life. Characteristic of shock is, not abnormal permeability of vessels, but avidity of tissues for fluids; therapy demands diffusible rather than nondiffusible fluid. 5. The most severe (especially intensive) shock proves fatal even when the blood is kept unconcentrated or slightly dilute by means of blood, plasma or a saline solution throughout. The conclusion favors a toxic cause not only of shock but also of the local exudation, on which the opposing physical hypothesis has been based.

DEVELOPMENT AND DEGREE OF SHOCK DETECTED BY SIMPLE MEANS. VIRGIL H. MOON, Jefferson Medical College.

Variations in arterial pressure are not an accurate means of recognizing shock nor of estimating its degree. Falls in arterial pressure result from various causes. Fall in pressure does not occur early in shock but only when the mechanism of compensation is failing. Shock may be well advanced while the blood pressure is at its highest recorded point. Hemoconcentration develops early, and its degree is commensurate with the gravity of the condition. It is etiologically related to the mechanism and pathologic aspects of shock, and it is easily determined. The technic of determining hemoconcentration is described, and the differentiation of shock from hemorrhage is discussed. A comparison of readings of blood pressure and of hemoconcentration in experimental and in clinical shock is presented.

EXPERIMENTAL STUDIES ON THE ROLE OF THE PRESSOR EPISODE IN THE ETIOLOGY OF ULCER. A. J. NEDZEL (by invitation) and WILLIAM F. PETERSEN, University of Illinois.

Geographically, ulcer of the stomach and of the duodenum is in general limited to the northern latitudes. Also, it most often involves the leptosome (a person of a slender, light physique); advancing vascular change also plays a role. So far as the oxidative demands of the mucosa of the intestine are great (a fact

established by Cohnheim many years ago) and vascular throttling by transient spasm, particularly in regions where other factors are of significance (e. g. that of a sphincter, that of peculiar distribution of the duodenal blood vessels and that of the lesser curvature, with its relatively poor anastomoses), obviously would lead to acute deficiency in the supply of oxygen, the great role of vascular disturbance in the pathogenesis of ulcer must be kept in mind. With acute deficiency in oxidation, necrosis of cells in the presence of an active digestive fluid must immediately lead to erosion and the formation of an ulcer. The observations of Konjetzny indicate that small lesions of the type to be anticipated are frequent in the mucosa of the ulcerous patient.

To reproduce the acute situation intravenous injections of pitressin (beta hypophamine) have been made in dogs (either of single or of repeated doses) and the effect of the injections has been studied directly in the mucosa of the living animals and, in addition, histologic material has been observed at varying intervals after such injections. In this material the gradation of change from the early vasomotor disturbance to the pronounced destruction observable in the old ulcer can be followed in detail. It is of interest to note that the number of ulcers produced by the injections of pitressin was greater in experimental animals observed during the spring than it was in those given injections during the autumn.

INFLUENCE OF SILICA ON NATURAL AND ON ACQUIRED RESISTANCE TO TUBERCULOSIS. ARTHUR J. VORWALD, Saranac Lake, N. Y.

The association of tuberculosis with silica is of such frequent occurrence as to indicate that silica in some way has a specific effect on the development of tuberculosis. The nature of this effect is still conjectural. Discussion concerning it ultimately resolves itself into two main categories: first, the influence of silica on tissue immunity; and second, the influence of silica on the growth and virulence of the infecting organisms.

The present investigations were concerned primarily with the factors of immunity to tuberculosis. They show that silica alters both the natural and the acquired resistance to tubercle bacillus infection. Animals that normally possessed a high innate ability to overcome a primary infection with organisms of either the attenuated or the more virulent type acquired progressive lesions when silica was combined with the infecting bacilli. This was demonstrated in guinea pigs after intraperitoneal injection of a suspension of attenuated tubercle bacilli of the human type plus silica and also in silicotic rats after infection with virulent organisms of the human type. With respect to acquired resistance, guinea pigs vaccinated with heat-killed organisms and later inoculated with silica in the right testis and virulent bacilli in the left testis manifested a resistance comparable to that in the control animals without silica. When the dust was combined with the sensitizing organisms and injected into the testis, the tuberculin skin tests remained strongly positive, but nevertheless the resistance to a test inoculation of virulent bacilli was markedly depressed. The last observations indicate that silica dust must be present in the immunizing focus to influence resistance to reinfection.

TISSUE CHANGES FOLLOWING PROLONGED ADMINISTRATION OF SULFANILAMIDE IN RATS. H. A. DAVIS (by invitation), L. C. HARRIS JR. (by invitation) and H. C. SCHMEISSER, University of Tennessee.

White albino rats weighing from 200 to 250 Gm. received daily subcutaneous injections of a 10 per cent suspension of sulfanilamide in sterile distilled water during a period of three months. The series was divided into four subgroups, each of which received, respectively, 0.5, 1, 1.5 and 2 Gm. of sulfanilamide per kilogram of body weight daily. Adequate controls were provided. The following tissue changes were observed: The blood revealed definite reduction in the number of erythrocytes and in the amount of hemoglobin and slight leukocytosis. In the liver there occurred diffuse degeneration of the parenchyma, focal necroses, and regeneration of liver cells, as evidenced by the number of mitoses. The spleen

revealed hyperplasia of the reticulo-endothelial cells, increased deposition of free iron, occasional focal necroses and, less commonly, myeloid metaplasia. In the kidney vacuolation and granularity of the tubular epithelium were present, but no specific glomerular changes. In one third of the treated animals definite bronchitis occurred, and sulfanilamide crystals could be observed in the bronchial exudate. The bone marrow of the femur showed mild hyperplasia of the myelopoietic and erythropoietic elements with an increase in megakaryocytes. The reticulo-endothelial system became definitely hyperplastic, chiefly in the spleen but also in the Kupffer cells of the liver, the reticulum of the lymphoid tissue and the macrophages of the general tissue. An increase of free iron was demonstrated by appropriate stains in the spleen, liver, kidney and adrenal glands. At the site of injection occurred proliferation of histiocytes, fibroblasts and numerous foreign body giant cells and, occasionally, necrosis of the skin. Deposits of sulfanilamide crystals were found in the capsules of the liver and spleen and in the meninges. In several animals proliferation of the endothelium of the blood vessels of the liver was present, forming small nodular projections into the lumen. Epistaxis and cyanosis of the buccal mucosa developed in several animals. An increasing tolerance to sulfanilamide could be readily produced. Unsuccessful attempts were made to obtain the Arthus phenomenon in the skin in order to determine the antigenic potentialities of sulfanilamide. The morphologic evidence of activation of the reticulo-endothelial system by sulfanilamide may provide a clue to its therapeutic activity.

EFFECT OF ANDROGENS ON EXOPHTHALMOS IN RABBITS. DAVID MARINE, Montefiore Hospital, New York.

Earlier observations showed that exophthalmos was more frequently produced in male rabbits, that castration caused its gradual regression and that cryptorchidism did not.

In the present experiments androgens have been used (testosterone propionate, androsterone and dehydro-androsterone). These substances increased or produced exophthalmos only in rabbits that had either had exophthalmos and recovered or had latent or mild exophthalmos. The reaction is greatest with testosterone propionate and least with dehydro-androsterone. In no instance was exophthalmos produced in rabbits that had never had exophthalmos, irrespective of thyroidectomy, thyroid feeding, cryptorchidism or gonadectomy, suggesting that other factors must be favorable in order to obtain exophthalmos with androgens. The data indicate that, in addition to thyroid insufficiency and increased pituitary activity, androgenic activity (active or passive) is also a factor favoring the development of exophthalmos. This factor is believed also to play a role in the percentile increase in the incidence of postthyroidectomy exophthalmos in exophthalmic goiter in males.

RADIOACTIVE IODINE AS A PHYSIOLOGIC INDICATOR IN STUDIES OF THE THYROID. SAUL HERTZ and ARTHUR ROBERTS (by invitation), Harvard Medical School.

In these studies use has been made of the previously known facts that iodine is selectively taken up by the thyroid gland when intravenously administered and that iodine can be made artificially radioactive in a variety of ways. By means of Geiger counters it is possible to detect minute quantities of iodine which has been rendered radioactive. The rabbit being used as a test animal, varying quantities of iodine have been injected intravenously, and by radiodetection methods the distribution of iodine in various parts of the treated animal has been studied. Results indicate that there is a selective concentration of iodine in the thyroid which is out of proportion to that found in various body fluids and other tissues.

The results show promise of application to beta irradiation of the thyroid in hyperplastic and neoplastic states when greater sources of radioactive iodine can be prepared by means of a cyclotron.

THE IODINE BALANCE IN DISEASES OF THE THYROID GLAND. ITALO D. PUPPEL (by invitation) and GEORGE M. CURTIS, Ohio State University.

Three normal persons who were maintained on a low intake of iodine, averaging 87 micrograms per three day period over a total period of twenty-four days, remained in continuous negative iodine balance. Three patients with exophthalmic goiter who were similarly maintained over a total period of thirty-three days revealed an increase in mobilization and an increase in circulation of iodine and an increase in excretion of the element through one or all excretory channels. Likewise, two patients with toxic nodular goiter who were investigated over a total period of twenty-seven days revealed an increase in excretion of iodine and a resulting profound disturbance of the iodine balance. One patient with carcinoma of the thyroid and pulmonary metastases who was investigated over a total period of thirty days showed a similar disturbance of the iodine balance. On the other hand, two patients with nontoxic nodular goiter who were investigated over a total period of thirty-six days remained in physiologic iodine balance.

In each instance, a constant regimen of experimental and hospital management was begun five or six days prior to the studies of iodine balance and was maintained throughout the period of investigation.

The effect of hospital management, of iodine therapy, of roentgenotherapy and of thyroidectomy on this increase in negative iodine balance of the patients with hyperthyroidism will also be presented.

INFLUENCE OF DIET ON LYMPHATIC TISSUES OF THE RABBIT. B. K. WISEMAN and JOSEPH H. AKEROYD (by invitation), Ohio State University.

The level of the lymphocytes in the peripheral blood and the reaction of the lymphoid tissues of the intestinal tract, of adjacent mesenteric nodes and of peripheral lymphoid deposits were studied in rabbits after introducing different food substances into the stomachs of different groups. Six animals received protein (a solution of egg albumin), six received carbohydrate (dextrose) and seven received fat (an emulsion of olive oil) exclusively. Vitamins and dextrose (the latter injected intravenously) were given to half the animals of all groups; all the animals received adequate amounts of essential minerals and water. In addition, controls on mixed diets and controls in a state of starvation were included. On each rabbit an average of thirty blood counts were made, and at the end of the experiment the tissues of the animal were examined supravitally and in paraffin sections. In general, the animals receiving fat and carbohydrate exclusively showed regressive changes in the lymphoid tissues, with lowered levels of lymphocytes circulating in the peripheral blood. Those receiving protein exclusively showed hyperplastic reactions, confined chiefly to the lymphoid tissue of the intestinal wall and mesenteric nodes; in addition, the lymphocytes of the peripheral blood reflected these stimulatory effects.

EFFECT OF THE PULSE ON THE FORMATION AND FLOW OF LYMPH. ROBERT J. PARSONS (by invitation) and PHILLIP D. MCMASTER, Rockefeller Institute for Medical Research, New York.

Experiments have been done to study the influence of the pulsation of blood vessels on the formation and flow of lymph and the spread of a dye (pontamine sky blue) through the interstitial tissues. Warmed oxygenated defibrinated rabbit's blood was perfused through the central artery of the rabbit's ear at constant pressures and at pulsatile pressures approximating those existing in a normal rabbit.

In the absence of pulsation of the blood vessels there was practically no formation or flow of lymph. The formation and flow of lymph were rapid when the perfused blood had a pulsatile flow. The rate of spread of the dye through the interstitial tissues was also increased under the same conditions. Edema occurred less frequently in ears through which blood was perfused at pulsatile pressures

than in those through which it was perfused at constant pressures. The appearance of edema in ears in which the perfused blood had a pulsating flow was accompanied by a conspicuous increase in the formation and flow of lymph. In the experiments with constant pressure the appearance of edema altered the formation and flow of lymph but little.

THE COMPARATIVE VALUES OF METHODS FOR DEMONSTRATING THE INCIDENCE OF TRICHINOSIS. LLOYD CATRON (by invitation), University of Michigan.

The following methods have been used to determine the incidence of infection by Trichinella spiralis in persons coming to autopsy at the University of Michigan Hospital: (1) microscopic examination of one section of diaphragm; (2) direct microscopic examination of 1 Gm. of finely ground diaphragm after thorough teasing or pressing between glass plates or both; (3) microscopic examination of the sediment which separates on standing after digestion of 50 Gm. of finely ground diaphragm in an aqueous solution of pepsin and hydrochloric acid. Thus far 160 diaphragms have been examined. One showed encysted larvae in the single section. Three showed larvae in 1 Gm. of ground tissue. Twenty-four showed them in the sediment obtained by digestion of 50 Gm. The corresponding percentages of cases in which these methods showed larvae are 0.62, 1.88 and 15. The digestion method demonstrated the larvae in all diaphragms in which larvae were disclosed by the other methods. In the instances in which larvae were found by direct examination of 1 Gm. of diaphragm, the larvae found numbered 1, 8 and 163 (in the last case they were also shown by method 1). Digestion in these instances yielded 3, 722 and 307 larvae. The last case, one of moderately severe infection in which all the larvae were dead and calcified, was the only one in which the digestion method compared unfavorably with direct microscopic examination. This resulted from failure of many of the calcified larvae to settle out on standing. In 20 of the 24 instances in which larvae were demonstrated by digestion, there were less than 30 larvae in 50 Gm. of diaphragm. In 2 of the 24 cases, part, and in 2 other cases, all of the larvae were dead and calcified.

INFLAMMATION OF SEROUS SURFACES: THE HYDROGEN ION CONCENTRATION IN RELATION TO THE CELL TYPE. BERNARD STEINBERG and ALBERT DIETZ (by invitation), Toledo Hospital, Toledo, Ohio.

This article will be published in full in a later issue of the ARCHIVES.

PHYSIOLOGIC PROPERTIES OF MONONUCLEAR PHAGOCYTES DERIVED FROM NORMAL AND FROM TUBERCULOUS ANIMALS. MAX B. LURIE, Henry Phipps Institute, Philadelphia.

An endeavor was made to elucidate the mechanism of the more rapid mobilization of mononuclear phagocytes which characterizes the response to reinfection in tuberculosis.

Supravital smears of exudative mononuclears obtained from normal and from tuberculous animals revealed that the macrophages of the latter are larger, contain more phagocytic material, show more numerous and larger vacuoles containing neutral red and exhibit more prominent and more actively motile pseudopodia than macrophages mobilized in normal animals under the same conditions.

The more rapid mobilization of mononuclears in the tuberculous animal, which occurs also in response to a nonspecific irritant, is associated with a greater incidence of amitotic cell division in the mononuclears than is observed in mononuclears from a normal animal. The phagocytosis of india ink by the mononuclears of tuberculous guinea pigs is more pronounced than that of normal animals. This can be demonstrated both in vivo and in vitro. Neither normal nor immune serum plays a role in this increased phagocytosis. It is a property of the macrophages derived from tuberculous animals. The increased phagocytic property of the macrophages of tuberculous rabbits can be demonstrated in vitro with carmine

particles and staphylococci. The heightened physiologic activity of the macrophages of tuberculous animals is in harmony with the acceleration noted in the sensitized animal of the succession of cells that characterize inflammation in general, i. e., the polymorphonuclear, mononuclear and lymphocyte sequence. It rationalizes the increased formation of hemolysin in tuberculous guinea pigs observed by Lewis and Loomis, and perhaps also the increased destruction or inhibition of tubercle bacilli phagocytosed in vivo by normal and "immune" mononuclears.

EFFECT OF THE COMPOSITION OF THE LIVER, AS INFLUENCED BY DIET, ON ITS SUSCEPTIBILITY TO INJURY BY CHLOROFORM. S. GOLDSCHMIDT (by invitation), H. M. VARS (by invitation) and I. S. RAVDIN, University of Pennsylvania.

The data to be presented constitute further proof, supported by analytic evidence, that the degree to which the liver cells of the rat suffer degenerative and necrotic changes when exposed to chloroform is proportional to the amount of lipid present in the liver.

The severity of the injury to the liver from chloroform is minimized after a high intake of carbohydrate, which results in a large and persistent deposit of glycogen in the liver. However, it has been found that equally large deposits of glycogen fail to protect the liver when but slightly more than minimal amounts of lipid are also present. The results show that the protective effect of a diet high in carbohydrate is chiefly to reduce the concentration of hepatic lipid and thus to lessen the amount of chloroform absorbed and the length of time it will exert its action on the cell protoplasm, leading to degeneration and necrosis.

THE LIFE CYCLE OF THE RED BLOOD CELL OF THE DOG. WILLIAM B. HAWKINS, University of Rochester.

Under standard conditions a healthy dog with biliary fistula eliminates daily a remarkably constant amount of bile pigment. It has been shown that the pigment radicle of destroyed hemoglobin is quantitatively eliminated as bile pigment. (A gram of hemoglobin is equivalent to 40 mg. of bile pigment.) By destruction of blood or by withdrawal of blood one can force this dog to fill its circulation with a great mass of new red cells and hemoglobin. Following this procedure, the output of bile pigment will fall, since fewer red cells are disintegrating daily. This decreased output will be maintained for many weeks, but subsequently there will be a conspicuous rise. The rise marks the obsolescence of the new red cells and determines the length of their life in a normal dog with biliary fistula. In four dogs the length of life of the red cell was determined as 112, 120, 126 and 133 days, or an average of 124 days.

On the basis of these figures, the calculated output of bile pigment averages 83 mg. per day and the observed output 85 mg. per day. This indicates that the bile pigment excreted daily is formed from destroyed red cells, with but little resulting from the "wear and tear" disintegration of muscle hemoglobin.

THE CELLULAR COMPOSITION OF THE BLOOD OF NORMAL RABBITS AS AN INDEX OF THEIR SUBSEQUENT REACTION TO EXPERIMENTAL SYPHILIS. ALBERT E. CASEY, PAUL D. ROSAHN and LOUISE PEARCE, St. Louis University, Yale University and Rockefeller Institute for Medical Research, respectively.

The average values for hemoglobin, erythrocytes, platelets, neutrophils, basophils, eosinophils, monocytes and lymphocytes were determined for the blood of normal rabbits. These rabbits were then inoculated with a strain of Spirochaeta pallida, and the incidence of primary lesions in the testes and of metastatic lesions in the bones and skin was determined. Significant relationships were noted between the preinoculation levels of the various blood elements and the subsequent reaction of the rabbits to experimental syphilis.

REGENERATION OF BLOOD PLASMA PROTEIN AS INFLUENCED BY AMINO-ACIDS. SIDNEY C. MADDEN (introduced by G. H. WHIPPLE), University of Rochester.

The regeneration of blood plasma protein in dogs is related to the quantity and quality of the protein in the diet. In the experiments reported, the dogs were standardized by reducing the protein in their blood plasma to a steady hypoproteinemic level of 4 per cent through daily plasmapheresis aided by their ingestion of a constant basal diet that was low but adequate in protein. It is assumed that the hypoproteinemia affords a maximal stimulus to regeneration of plasma protein. The standardization required from four to eight weeks. It was found empirically that during the initial two to six weeks more plasma protein had to be removed per day (reserve store) than it was subsequently necessary to remove (basal output) in maintaining the steady hypoproteinemia. When this constant minimal basal output was reached, controlled variations were introduced. When gelatin was added to the basal diet, a slightly increased amount of plasma protein had to be removed to keep the concentration of the circulating protein at 4 per cent. When cystine accompanied by either tryptophane or tyrosine or both was added with the gelatin, a greatly increased amount of plasma protein had to be withdrawn to maintain standard conditions. This increase was sometimes five times that occasioned by the simple addition of gelatin. On the other hand, cystine alone and trytophane and tyrosine alone or together were ineffective in increasing the output over that attributable to gelatin.

ACUTE AND CHRONIC HYPOPROTEINEMIA INDUCED BY INJECTIONS OF ACACIA. RALPH E. KNUTTI, University of Rochester.

A single intravenous injection of a solution of acacia in a dog is followed by a fall in the total plasma protein and in the fibrinogen. This fall is in part associated with the dilution of the plasma but is chiefly due to actual loss of plasma protein.

It is possible to maintain dogs at low levels of total plasma protein and of fibrinogen for periods of months by repeated injections of the solution of acacia. If these injections are discontinued in such animals, the plasma protein and fibrinogen remain low for many weeks. At the same time acacia persists in the blood, gradually diminishing as the plasma proteins gradually rise. It appears that dogs with Eck fistulas respond more readily to this "acacia effect" than do normal dogs. Since the anatomic changes following such a procedure are most conspicuous in the liver, these observations further implicate the liver as a source of plasma protein.

RESTRICTION OF THE REGENERATION OF HEMOGLOBIN IN ANEMIA BY A PROTEIN-DEFICIENT DIET. PAUL F. HAHN (introduced by G. H. WHIPPLE), University of Rochester.

Of the three essential constituents of hemoglobin, at least two, if the supply is inadequate, may limit the production of hemoglobin in anemia. The body has been shown capable of producing a supply of the pyrrole aggregate (pigment nucleus) ample to meet almost any emergency. The restrictive effect of a deficiency in iron has long been recognized. The need of the globin fraction of hemoglobin, even though it is usually obscured by the deficiency in iron, is nevertheless a real one. An anemic animal maintained on a diet lacking iron but otherwise ample may show an increase in hemoglobin of from 50 to 75 Gm. per week when the needed iron is supplied. However, when in anemia the intake of protein is reduced to a point below the sum of the amounts needed for ordinary metabolism and for production of hemoglobin there is restriction of the production of hemoglobin in spite of excess administration of iron.

QUANTITATIVE ESTIMATION OF ASH IN HISTO-INCINERATED TISSUE SECTIONS: THE PHOTO-ELECTRIC METHOD. GEORGE ZUR WILLIAMS (introduced by Frank L. Apperly), Medical College of Virginia, Richmond.

The increasing use of histo-incineration methods for the disclosure of the distribution of ash in experimental studies of tissues makes desirable the development of a method which is at once rapid, accurate and highly sensitive for the quantitative estimation of ash in tissue sections. The customary method has been by visual approximation with dark field illumination or by comparison of photomicrographs. In comparison with quantitative chemical estimation of calcium and silica such crude estimation is found unreliable.

A very sensitive and reliable photo-electric method for the estimation of the amount of light reflected from particles of ash in histo-incinerated sections is described. Methods of standardization and control experiments are briefly discussed, particularly distribution curves for particle size and number in relation to amount of light reflected.

This standardized equipment is being used in the study of quantitative determinations of ash in normal and in tumor tissue after irradiation. Reference is made to a similar method reported by Scott.

A COMPARISON OF THE EFFECT OF TOTAL HEPATECTOMY WITH THAT OF INTRA-ABDOMINAL EVISCERATION ON THE BLOOD LIPIDS OF THE DOG. STEPHEN MADDOCK, Boston City Hospital.

Further studies of the effect of total removal of the liver on the blood lipids of the dog revealed that the disappearance of the blood fats cannot be accounted for by this experiment alone. A study has also been made of the animal in which all of the abdominal viscera, including the liver, have been removed and also on the animal in which the liver has been left behind, with its circulation intact, but from which the remaining intra-abdominal viscera have been removed. Experiments have also been made to determine the effect of intravenous dextrose on the blood lipid content.

EFFECT OF CERTAIN SUGARS ON THE HYDROGEN ION CONCENTRATION AND THE LACTIC ACID CONTENT OF WALKER SARCOMA 319. FRANCES F. BECK (by invitation), RUTH MUSSER (by invitation) and JOHN C. KRANTZ JR., University of Maryland School of Medicine.

The studies were made with a glass electrode inserted directly into the tissues. The lactic acid was determined. Intraperitoneal injections of dextrose, dihydroxyacetone and hydroxypyruvic aldehyde lowered the hydrogen ion concentration and increased the lactic acid content. Dextrose had no effect on the hydrogen ion concentration of muscle, while dihydroxyacetone and hydroxypyruvic aldehyde lowered it. All three sugars increased the lactic acid content of muscle.

INFLUENCE OF CERTAIN INORGANIC SALTS ON TUMOR GROWTH IN THE RAT. R. L. FERGUSON (by invitation), MARY C. PATRAS (by invitation) and R. D. TEMPLETON (introduced by PAUL R. CANNON), Loyola University School of Medicine, Chicago.

One hundred and fourteen male albino rats from 21 to 31 days of age were distributed by litter mates into four groups. The first group received a diet consisting of casein 18 per cent, starch 47, butter 15, yeast 15, cod liver oil 2 and an inorganic salt mixture (Harris) 3 per cent. The second group received a salt-deficient diet, which was made by substituting starch for the salt in the aforementioned diet. The third and fourth groups received 2 per cent calcium hydroxide and 0.05 per cent cobalt sulfate, respectively. These diets were made by substituting the desired quantities of the salts for their equivalents of starch in the salt-deficient diet.

Each rat was bilaterally inoculated with tumor tissue (spindle cell sarcoma) after being on its respective diet one week. The influence of the diets on tumor growth was roughly proportional to their influence on body weight.

EXPERIMENTAL OBSERVATIONS ON THE MECHANISM OF ACHLORHYDRIA IN GASTRIC CARCINOMA. ALEXANDER BRUNSCHWIG and JOHN VAN PROHASKA (by invitation), University of Chicago.

Extracts of three human carcinomas and of the adjacent pyloric mucosas in cases in which there was achlorhydria were made with boiling 0.4 per cent hydrochloric acid. These extracts were filtered and neutralized and then injected intravenously into dogs with subtotal gastric pouches. "Foreign protein" reactions occurred, with hyperpyrexia, and were accompanied over a period by marked suppression of gastric secretion and achlorhydria. Other experiments showed that hyperpyrexia due to injection of bacterial proteins was accompanied by a similar reaction. After immunization to the latter proteins such injections produced no achlorhydria and no suppression of gastric secretion. Repeated injections of the extracts resulted in immunization so that no hyperpyretic reactions were obtained, but suppression of secretion and achlorhydria continued to be produced. Injections of extracts of carcinoma of the human colon, carcinoma of the breast, human goiter and normal human pyloric, fundic, esophageal, jejunal, ileal and colon mucosa did not at any time produce a "foreign protein" reaction or suppress gastric secretion or affect the acidity. Injection of extracts of metastases from a large primary growth which was not accompanied by achlorhydria did not produce a reaction or affect the gastric acidity. This report is preliminary, but it suggests that achlorhydria in gastric carcinoma may be due to some substance developed in the stomach or in the growth incident to the development of the latter. The experiments are being continued.

THE SERUM LIPIDS AND THE OSSEOUS METABOLISM IN PRIMARY CARCINOMA OF THE LIVER. A. E. HANSEN (by invitation), M. R. ZIEGLER (by invitation) and IRVINE MCQUARRIE, University of Minnesota.

The development of generalized osteoporosis of an extreme degree in an 11 year old boy suffering from primary carcinoma of the liver suggested the desirability of an investigation of the obvious disturbances in his metabolism. While the calcium, inorganic phosphorus and phosphatase activity of the serum were normal, the patient was found to retain less than a third as much calcium and phosphorus as a normal subject on the same diet. Retention of these elements could be increased temporarily by greatly increasing the intake of them, but the excess stored was lost promptly on resumption of a normal diet. This and other observations suggest the possibility that the liver plays an essential role in bone metabolism.

A generalized disturbance of the lipid metabolism was indicated by a gradual increase in the degree of lipemia, involving all fractions of the blood lipids. The total plasma cholesterol reached a concentration of 1,200 mg. and the lecithin 1,000 mg. per hundred cubic centimeters. The iodine number of the serum fatty acids was extremely low. The spleen and other organs showed the presence of many cells filled with lipoid materials, a circumstance which was regarded as being secondary to the prolonged lipemia. These observations are interpreted as confirming the theory that the liver plays an essential role in lipid metabolism.

PATHOLOGY OF AN ADENOMATOUS LESION OF THE PYLORIC CHAMBER OF THE STOMACH IN MICE OF STRAIN I. HAROLD L. STEWART and H. B. ANDERVONT, Harvard University Medical School.

In mice of strain I the pyloric chamber of the stomach, which is normally lined by glandular mucosa, undergoes extensive adenomatous hyperplasia. The origins of this lesion are noted before the age of 3 months, and by the ninth

month of life the stomachs of all animals of this strain show the process well developed. The glands in the gastric mucosa dilate, the lining cells become irregular in size, shape and staining, and cellular division goes on at a rapid rate. These hyperplastic changes lead to constriction of the gastric lumen and pronounced thickening of the pyloric chamber, which eventually constitutes the main body of the stomach; the cardiac chamber shows little or no thickening but is usually compressed. To date the process has not metastasized, and no infectious agent has been constantly demonstrated in association with it. Many mice of strain I die of starvation, anemia or gastric hemorrhage before the fourteenth month.

INHIBITIVE INFLUENCE OF TRANSPLANTED LEUKEMIC LYMPHOID CELLS ON THE METABOLISM OF NORMAL TISSUE. JOSEPH VICTOR and JAMES S. POTTER, College of Physicians and Surgeons, Columbia University, New York, and Carnegie Institution of Washington, Cold Spring Harbour, Long Island, N. Y.

The intraperitoneal inoculation of a saline suspension of leukemic spleen or lymph nodes depresses the metabolism of uninfiltrated lymphoid tissue of the host. The leukemic cells of two different mouse transmission lines depressed the anaerobic glycolytic rates but not the respiratory nor aerobic glycolytic rates of uninfiltrated tissue. Similar suspensions of normal spleens of two different strains of mice produced no such effect. The inoculation of spleen of a foreign strain (Storrs-Little) into C58 mice increased the glycolytic rates of C58 lymphoid tissue, while similar treatment with tissue of the same strain had no effect. No metabolic inhibition occurred when normal and leukemic lymphoid cells were mixed in Ringer solution; nor could any inhibitory substance be demonstrated in Ringer solution in which leukemic cells had been suspended. It is concluded that the depression of the anaerobic glycolysis of uninfiltrated lymphoid tissue in transmissible leukemia is due to a humoral factor which results from the reaction between the host and the leukemic cells.

ISOLATION AND IDENTIFICATION OF THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS. MARION E. HOWARD (introduced by FRANCIS G. BLAKE), Yale University.

A strain of the virus of lymphocytic choriomeningitis, isolated from the spinal fluid of a human patient, has been passed through mice for forty-four generations. The results of experimental reinoculations relate it to the Rivers W. E. strain of the virus. Neutralizing antibodies in the patient's serum appeared several months after the acute infection. Serum-protected mice are not immune to infection by reinoculation.

COOPERATIVE ACTION OF HAEMOPHILUS GALLINARUM AND THE COCCOBACILLIFORM BODIES IN FOWL CORYZA. JOHN B. NELSON, Rockefeller Institute for Medical Research, Princeton, N. J.

Fowl coryza is divisible into several types, which differ in the length of the incubation period and the duration of the attendant nasal discharge. This report is concerned with the etiology of type III, a coryza of rapid onset (from one to three days) and long duration (two months or more), with which Haemophilus gallinarum is always associated. This bacterium is infective for normal birds but produces in them a coryza of short duration which fails to protect them, on recovery, against the natural disease.

It was found that coccobacilliform bodies identical with those which cause another type of coryza are also associated with type III and are demonstrable together with H. gallinarum throughout the entire course of the illness. It was known that the specific bodies, injected in pure culture, are rarely demonstrable before the second week, and H. galiinarum, after the second week. The injection of a mixture, however, accelerates the growth of the former and prolongs the residence of the latter, resulting in a coryza which closely approximates the natural disease. When present together, the two infective agents provoke a reaction which

neither is able to provoke alone. This synergistic relation between H. gallinarum and the coccobacilliform bodies offers an adequate etiologic explanation of coryza of type III.

BIRDS AS POSSIBLE CARRIERS OF THE VIRUS OF EQUINE ENCEPHALOMYELITIS. CARL TEN BROECK, Rockefeller Institute for Medical Research, Princeton, N. J.

The epidemiology of equine encephalomyelitis suggests that there may be a reservoir host from which mosquitoes secure virus and suggests further that this host may be a bird. Therefore the reactions of chickens and turkeys to the virus have been studied. Neither the chicken nor the turkey shows any clinical evidence of disease after a superficial injection of a small amount of virus, but both show a high level of virus in the blood for several days, followed by the appearance of neutralizing antibodies. Serums from domestic fowls on farms where the disease occurred have been tested for neutralizing antibodies, and in a few instances these bodies have been found. The results indicate that domestic and probably wild birds may be infected and that birds may play a part in the transmission of the disease.

SEROLOGIC EVIDENCE FOR THE OCCURRENCE OF INFECTION WITH THE VIRUS OF HUMAN INFLUENZA IN SWINE. RICHARD E. SHOPE, Rockefeller Institute for Medical Research, Princeton, N. J.

Antibodies capable of neutralizing the virus of human influenza were present in the serums of old swine on the farm of two New Jersey institutions but absent from the serums of young swine on the same farms. The old animals had lived through the winter of 1936-1937, in which outbreaks of disease of the upper respiratory tract were prevalent among the inmates of the two institutions, while the young swine studied were born long after these outbreaks. It is believed that the swine the serum of which neutralized the virus had undergone an unrecognized infection with influenza virus acquired from man.

EXPERIMENTAL CARBONTETRACHLORIDE CIRRHOSIS. JESSE L. BOLLMAN and FRANK C. MANN, Mayo Foundation, Rochester, Minn.

Acute intoxication with carbontetrachloride produces central necrosis of the liver. Repair of the injured tissue is complete in about two weeks. Repeated administration of carbontetrachloride at intervals before the regeneration of the liver is complete produces distortion of the hepatic architecture typical of the portal type of cirrhosis. Impairment of hepatic function may be indicated by jaundice, ascites, alterations of the serum proteins and other conditions. The extent of the lesion depends on the nutritive condition of the liver at the time the drug is given. If the administration of carbontetrachloride is discontinued when extensive cirrhosis has been produced there is gradual disappearance of symptoms and return of hepatic function.

PATHOLOGIC EFFECTS OF CERTAIN GLYCOLS AND RELATED COMPOUNDS. H. D. KESTEN, M. G. MULINOS and LEO POMERANTZ (by invitation), College of Physicians and Surgeons, Columbia University.

The mono-ethyl, monomethyl and monobutyl ethers of diethylene glycol, as well as diethylene glycol itself, when administered to rats and rabbits by mouth or intravenously in adequate dosage, cause extensive hydropic degeneration and destruction of renal tubular epithelium, leading to uremia. The liver and adrenal cortex are also often involved.

Dipropylene glycol produces similar injuries but less regularly and only with higher dosage.

Diethylene dioxide (dioxane) produces lesions in the kidneys and liver similar to those caused by diethylene glycol and its derivatives.

The administration of ethylene glycol leads to blockage of renal tubules by crystalline masses (oxalates?) with minimal epithelial degeneration.

Propylene glycol given orally to rats is relatively innocuous. Given intravenously in large doses to rabbits, it produces hemoglobin casts in the renal cortex.

Of the chemicals thus far used, only those containing one or more ether linkages cause hydropic degeneration of parenchymatous epithelial cells.

EXCRETION OF WATER, CYANOL AND AZOFUCHSIN I IN EXPERIMENTAL GLOMERULAR NEPHRITIS. WILLIAM E. EHRICH (introduced by E. B. KRUMBHAAR), University of Pennsylvania.

Glomerular nephritis was produced according to the method of Masugi by immunizing ducks with rabbit kidney and by injecting the antikidney serum into rabbits. The dye cyanol was used to test glomerular filtration; azofuchsin I, to test tubular secretion. The glomerular nephritis produced appeared to be the same as human glomerular nephritis. As to the tests, during the acute phase of the disease the excretion of water and of cyanol was diminished, whereas the excretion of azofuchsin I was not changed. Since during this time the glomeruli were solely or mainly involved, and as but one animal showed marked tubular involvement, and since this animal was the only one which also showed a decrease in excretion of azofuchsin I, it is concluded that glomeruli and tubules may function independently of one another.

A NONSPECIFIC FACTOR IN IMMUNITY TO PNEUMOCOCCUS. HOBART A. REIMANN, Jefferson Hospital, Philadelphia.

Several years ago I showed that an increased viscosity of the blood incident to an increase in the amount of globulin in the blood, such as occurs during certain infectious diseases, markedly enhances the agglutination of bacteria when specific immune serum is present in weak dilution. Similar enhancement could be shown in vitro by the use of solutions of acacia of different viscosities, but not by the use of gelatin, starch or egg albumin. An optimal point of viscosity was found, which was similar to that attained by the blood when hyperinosis is present as found in infection, beyond which agglutination is hindered. Experiments were then made to determine whether or not changes in the viscosity of blood plasma or of acacia solution modified the nature or the degree of swelling of the capsules of pneumococci in the presence of weak dilutions of specific immune serum. Definite changes were found in viscous solutions as compared with salt solution or broth. Since agglutination of pneumococci and swelling of their capsules have been found in patients treated with antipneumococcus serum, it is suggested that the nonspecific influence of globulins in enhancing these changes may be a factor in immunity.

EARLY CHANGES IN THE LUNG INDUCED BY RADIATION. SHIELDS WARREN, Harvard University Medical School.

Therapeutic irradiation of carcinoma of the human breast or of the esophagus may produce clinically demonstrable changes in the lung. Study of lungs removed at autopsies following varying amounts of irradiation of the chest permitted following the sequence of changes in the alveolar lining cells, the alveolar walls and the vascular bed. The observations have been paralleled by others on a series of experimentally irradiated rat and rabbit lungs.

The early stages are represented by changes in the alveolar lining cells; the late, by interstitial fibrosis and thickening of the walls of blood vessels.

NEGATIVE CHEMOTROPISM IN LEUKOCYTES. MORTON MCCUTCHEON and DALE REX COMAN (by invitation) and H. M. DIXON, University of Pennsylvania Medical School.

The outcome of an infection—whether recovery or death—may depend on the ability of the leukocytes to display positive chemotropism, i. e., to move toward the infecting bacteria, thus making possible phagocytosis and destruction of the bacteria.

If leukocytes are unable to approach the source of infection, or if they are actually repelled so that they move in the opposite direction, i. e., display negative chemotropism, phagocytosis cannot occur. It is therefore of interest to ascertain under what conditions, if any, leukocytes exhibit negative chemotropism. A survey of the literature shows that there have been few if any reports of instances in which under experimental conditions leukocytes were actually repelled by bacteria or substances of any kind, and that while the terms "negative chemotropism" or "negative chemotaxis" are often used, they indicate, as a rule, merely absence of attraction rather than repulsion.

Our purpose in the present paper is to record the results of experiments in which definite repulsion of leukocytes was induced. The cells were obtained from peritoneal exudates of rabbits and were suspended in the plasma of the rabbit. The substance to be tested was suspended in distilled water, and a drop of this suspension was placed on a glass slide, where it dried, forming an object about 0.1 mm. in diameter. A drop of cell suspension on a cover glass was superimposed on the test object. The preparation was sealed with petrolatum and examined at 37.5 C. with the microscope. With the help of a drawing ocular, a record was made of the path of each leukocyte as it moved toward or away from the test object. The paths of many leukocytes were analyzed to determine the average reaction of the cells.

Definite repulsion of rabbit leukocytes was brought about by kaolin and by a chemically related preparation, Lloyd's reagent (a specially prepared fullers' earth). Both these substances are aluminium silicates. The size of the particles was approximately that of ordinary bacteria.

Negative chemotropism was also produced by a strain of the beta hemolytic streptococcus in some of the preparations, while in others positive attraction was observed. The variable response of leukocytes to this organism is being investigated further.

PRODUCTION OF MACROCYTIC ANEMIA IN THE PREGNANT RAT BY DIETS LOW IN PROTEIN. JEAN KYER (by invitation) and FRANK H. BETHELL, Simpson Memorial Institute, University of Michigan.

The effects of a number of diets supplying variable amounts of protein were studied with regard to the blood changes occurring during pregnancy. Of primary concern were the number and hematocrit percental volume of the red blood cells, the hemoglobin value and the mean corpuscular volume.

Diets composed either of naturally occurring foods or of purified materials were fed to female rats for at least seven days before breeding and throughout the period of gestation. In those receiving 20 per cent or more of their food as animal protein the blood values were maintained practically unchanged throughout pregnancy. A protein content of 10 per cent or less led to a reduction in the red blood cell count of variable degree, a less marked decrease in hemoglobin and an increase in the size of the erythrocytes, giving the picture of a relatively severe macrocytic anemia. The values obtained on diets high in protein were as follows: red blood cells, from 7,300,000 to 10,500,000 per cubic millimeter, with an average of 8,500,000; hemoglobin, from 12.3 to 17.2 Gm. per hundred cubic centimeters, with an average of 13.7 Gm.; hematocrit percental volume, from 36 to 50, with an average of 41.7; mean corpuscular volume, from 56 to 43 cubic microns, with an average of 49. On diets low in protein the values were: red blood cells, from 2,700,000 to 7,900,000, with an average of 5,600,000; hemoglobin, from 5.6 to 12.7 Gm., with an average of 11.7 Gm.; hematocrit percental volume, from 19 to 42, with an average of 34.6; mean corpuscular volume, from 87 to 52 cubic microns, with an average of 64.

DEVELOPMENT OF CIRRHOSIS IN FATTY LIVERS. CHARLES L. CONNOR and I. L. CHAIKOFF (by invitation), University of California.

The present report deals with a continuation of previous work in which cirrhosis of the liver was demonstrated as an end-result of long-continued fatty

infiltration of the liver in depancreatized dogs kept alive for from 2.5 to 5.6 years. These dogs were maintained with insulin and a diet adequate in calories, proteins and vitamins but containing no raw pancreas. Cirrhosis appeared in some animals as early as 2.5 years after pancreatectomy at a time when large amounts of fat were still present in the liver, while in a number of animals examined between 4.2 and 5.5 years after pancreatectomy the fat had disappeared and the cirrhosis was the only lesion present. The condition of these livers seemed identical with the cirrhosis found associated with alcoholism and with that found in patients suffering from diabetes mellitus. A study is now being made of the effects of alcohol on livers which had been made fatty by previous feeding with a diet high in fat. Preliminary experiments have resulted in the production of the first stages of this disease in dogs. A few examples from human sources illustrate again how cirrhosis may follow fatty infiltration resulting from alcoholism.

EXPERIMENTS IN TRANSMISSION OF CARCINOMA IN FROGS. BALDWIN LUCKÉ and H. SCHLUMBERGER (by invitation), University of Pennsylvania.

In previous experiments it has been found that when the renal tumor of the leopard frog (adenocarcinoma with intranuclear inclusions) is inoculated intramuscularly, intracranially or into the lymph sacs or the celomic cavity a tumor rarely appears at the site of inoculation and that if one does it will soon retrogress, but that, in the kidney, carcinoma appears in about a fourth of the frogs surviving inoculation for more than one year. This occurs regardless of whether living tumor cells (solid tumor grafts or a suspension of the tumor cells) or desiccates of tumors are used. These experiments are interpreted as indicating the existence of an organ-specific factor, probably a virus.

The present experiments deal with inoculation, by surgical operation, of tumor grafts into the kidney, liver and eye. When inoculation was made directly into the kidney, a tumor developed in this organ in about a third of the animals which survived for six months. After inoculation in the liver no significant local growth occurred, but in the kidneys of the animal thus inoculated tumors developed also in about a third of those surviving for six months. Transplants into eyes grew well in a high proportion of the frogs; in this series, too, renal tumors were frequent. The transplantation of the tumor into the chambers of the eye proved an excellent method to use for continued observation of local tumor growth.

The most acceptable explanation of these experiments is that the tumors are caused by a virus which is specific for the kidney.

PHOTO-OXIDATION OF ANTIGEN AND ANTIBODIES. HANS SMETANA and DAVID SHEMIN (by invitation), College of Physicians and Surgeons, Columbia University.

Antigen (crystalline egg albumin) exposed to visible light in the presence of hematoporphyrin loses its antigenicity and can no longer be precipitated by specific antiserum. Control solutions remain active.

The amount of oxygen taken up by the acceptor-dye compound during exposure to light until the precipitin reaction is extinguished is constant for the same antiserum but varies for different antiserums. Injections of the inactivated antigen no longer produce antibodies in rabbits.

Egg albumin antiserums lose their specific precipitating action after exposure to light in the presence of hematoporphyrin while control solutions remain active. The amount of oxygen taken up by the serum-hematoporphyrin compound during exposure to light until the precipitating action is destroyed is small.

Chemical investigations of the photo-oxidized proteins and amino-acids were undertaken with a view to studying the changes which occurred in these substances as a result of the treatment described. Changes in the amino-acid content, aminonitrogen and noncoagulable nitrogen and the attack on various groupings of proteins were studied.

CONSTITUTIONAL (INHERITED) RACHITIC-LIKE DISORDERS IN THE RABBIT. LOUISE PEARCE, Rockefeller Institute for Medical Research, Princeton, N. J.

In a rabbit breeding colony assembled for the purpose of studying constitutional and environmental factors in disease, abnormalities of the bones which resemble human rickets have been observed in racial lines representing six breeds. Each of these groups differs in the distribution of lesions and resulting deformities, in some respects, from the others. In several groups the axial as well as the appendicular skeleton is affected.

Preliminary observations indicate that the predisposition to the occurrence of the abnormalities is definitely inherited. Intensive breeding experiments directed to the fixation of the character have been carried out, and in one racial line of the Beveren breed the abnormality of the forelegs is now of an extreme degree.

The condition can be detected as early as the tenth day and begins clinically with enlargement of the distal ends of the radius and ulna and a suggestion of a flare or flipper position of the paws. The deformity increases rapidly and progressively, and by the second month both bones show extreme medial curvature. There is a variable change in the anterior-posterior curve of the bones, but in many instances the curve, particularly of the radius, is pronounced.

Microscopic and roentgenographic studies show that the condition strikingly resembles human rickets. Chemical studies of the blood and experiments on the effect of the administration of vitamins and of calcium and phosphorus to the mother and nursing young are in progress, while other experiments dealing with the effect of various light environments will shortly be carried out.

The nature of the hereditary factor is now being investigated by appropriate breeding tests. The preliminary results show that the condition regularly appears in the F_1 generation, although in only a moderate degree, while in backcross progeny it is marked. Other evidence suggests the cumulative nature of the factor, but experiments designed to elucidate this point are difficult to make because of a lowered fertility rate.

At one time, heredity was regarded as a factor of more or less importance in human rickets, but with the discovery of the efficacy of dietary and hygienic measures the idea of a hereditary influence was discarded despite the evident racial factor in the disease. The evidence now available indicates that heredity is a potent factor.

HEREDITARY BRACHYDACTYLIA AND ALLIED DEFORMITIES IN THE RABBIT. HARRY S. N. GREENE and JOHN A. SEXTON JR. (by invitation), Rockefeller Institute for Medical Research, Princeton, N. J.

The hereditary nature of brachydactylia and more extensive deformities of the feet of man has long been recognized, but investigation of their pathogenesis and of the relationship of the different types has been limited by a lack of experimental material. The occurrence of a comparable series of abnormalities in rabbits offers an approach to such problems.

The deformities in rabbits range from slight digital shortening to complete acheiropodia, but genetic studies show that the different types are interrelated, each being a modified form of the same fundamental variation. The occurrence of the variation is dependent on a single recessive unit factor, while the type is determined by the action of supplementary modifiers.

The deformities are present at birth, and degenerative changes in the foot anlage have been found on the sixteenth day of intra-uterine life. Venous stasis with hemorrhage and necrosis of neighboring parts is a constant finding in affected embryos, and study of a large group indicates that the primary site of variation is the vascular system.

Sex and Age Differences in the Anterior Lobes of the Pituitary Glands of Rabbits as Determined by Implantations in Guinea Pigs. John A. Saxton Jr. (by invitation) and Harry S. N. Greene, Rockefeller Institute for Medical Research, Princeton, N. J.

In a rabbit breeding colony devoted to the investigation of constitutional and environmental factors in disease, studies of endocrine physiology are being carried out. The work has included a study of the anterior lobe of the pituitary gland by the implantation technic, with use of the immature female guinea pig as the test animal. One phase of the study has dealt with the factors of sex and age.

Anterior lobes of pituitary glands obtained from mature rabbits were individually analyzed by implantation of one-quarter gland daily. Definite differences in the response of the ovaries and adrenal glands of the test animals were observed with respect to the sex of the rabbits from which the anterior lobes were obtained. Those from male rabbits exerted stronger gonadotropic effects, both follicle stimulating and luteinizing, and also induced greater proliferation of the adrenal cortex.

Anterior lobes of pituitaries obtained from immature rabbits (from 1 to 10 weeks of age) were similarly analyzed, a small group of glands obtained from rabbits of the same age being used for each test animal. No gonadotropic effect was found after implantation of anterior lobes from 1 week old rabbits. Those from 2 week old rabbits produced only activity of the follicles, while those from older immature rabbits produced luteinization as well as activity of the follicles.

Studies of the Bone Marrow and Liver in Experimental Anemia of Pregnancy. Frank H. Bethell, University of Michigan.

The total number of nucleated cells in the marrow of the rat's femur is not greatly altered during pregnancy regardless of the protein content of the diet. The number varies between 1,500,000 and 2,500,000 per cubic millimeter. Of these, a little over 50 per cent are cells of the leukocyte series. The remainder may be divided into four groups: primitive blasts (believed to be chiefly erythroblasts), megaloblasts, basophilic normoblasts and eosinophilic normoblasts.

Rats receiving an inadequate amount of protein during pregnancy acquire, in conjunction with macrocytic anemia, relative and absolute megaloblastosis, the increase amounting to about 500 per cent of the number found in the marrow of pregnant rats maintained on a diet high in protein. Coincident with the changes in the blood and bone marrow there is marked lipoidosis of the central portion of the hepatic lobules. Animals maintained on diets low in protein for long periods of time may in the absence of pregnancy present similar changes, but to a less marked degree.

The Bleeding Tendency and the Level of Prothrombin in Dogs with Biliary Fistula: Bile and Alfalfa (Vitamin K) Feeding. K. M. Brinkhous (by invitation), E. D. Warner, W. H. Seegers (by invitation) and H. P. Smith, University of Iowa.

It has been shown by Hawkins and Brinkhous that the bleeding tendency in dogs with biliary fistula is due to a low level of prothrombin in the plasma. Feeding of whole bile prevented the fall in prothrombin. We have fed bile or bile salts to dogs deficient in prothrombin and have found that the prothrombin increases slowly over a rather long period. If, in addition to bile, a fat-soluble extract of alfalfa meal, rich in vitamin K, is fed, the level of prothrombin rises much more rapidly.

Effect of Substances of the Sulfanilamide Group on the Chemotropism of Leukocytes. Dale Rex Coman (introduced by Isolde Zeckwer), University of Pennsylvania Medical School.

The purpose of these experiments was to determine whether or not substances of the sulfanilamide group increase chemotropism of leukocytes and in this way exert their therapeutic action.

The chemotropic effects of prontosil (a derivative of sulfanilamide, known as prontosil-soluble [the disodium salt of 4-sulfamidophenyl-1-2′-azo-7′-acetylamino-1′-hydroxynaphthalene-3′, 6′-disulfonic acid]), of sulfanilamide and of setazine were studied by noting the response of rabbit polymorphonuclear leukocytes to these substances in vitro. Prontosil-soluble was adsorbed on kaolin, carbon and hemolytic streptococci, respectively. Control preparations of kaolin, carbon and streptococci were also studied. Sulfanilamide and setazine were used in crystalline form. The paths of leukocytes were followed under the microscope, as they moved in relation to these substances. The measure of chemotropism adopted was the number of microns per minute the cells moved toward the test substance. Prontosil did not significantly alter the chemotropic response of the leukocytes. Sulfanilamide in the concentration employed resulted in a cessation of cell movement. Setazine caused only slight increase in chemotropism.

From these experiments it is considered unlikely that the therapeutic effect of these compounds is due to an increase produced in the chemotropism of leukocytes.

COMMENTS ON THE COLORIMETRY INVOLVED IN THE ASSAY OF SULFANILAMIDE. MICHEL PIJOAN (introduced by ELLIOTT C. CUTLER), Peter Bent Brigham Hospital, Boston.

The purpose of this communication is to examine the intrinsic colorimetry involved in the assay of sulfanilamide and to determine if the final chromogen content of the diazodye is proportional to the sulfanilamide present.

My associates and I used various methods but especially the one presented by Marshall, Emerson and Cultruy, in which the final pigment produced has a simple broad absorption band at 524 microns. Additional experiments reveal that the optical densities of the resulting chromogen are directly proportional to the amounts of the sulfanilamide added. The spectrophotometric studies were also supported by experiments using the Evelyn photometric colorimeter with a Corning glass filter transmitting a narrow spectral band in the vicinity of 524 microns. By this method interfering colors, such as the yellow in blood pigments, were excluded. The relationship of sulfanilamide to the resulting chromogen is stoechiometric.

NEW YORK PATHOLOGICAL SOCIETY

N. CHANDLER FOOT, *President*

Regular Monthly Meeting, Dec. 23, 1937

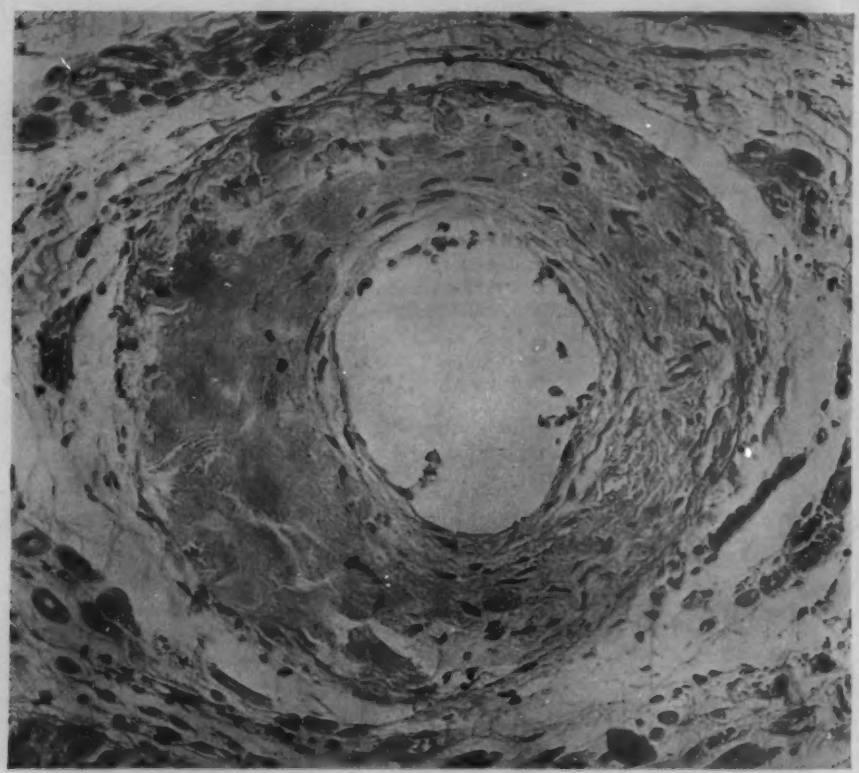
MILTON HELPERN, *Secretary*

GENERALIZED ARTERIOSCLEROSIS WITH HYPERTROPHY OF THE MEDIA AND ATYPICAL HYALINE DEGENERATION AFFECTING THE INTIMA, MEDIA AND ADVENTITIA. CHARLES T. OLCOTT.

A white mechanic 66 years old had had frequent attacks of "bronchial asthma," which followed acute laryngitis with aphonia thirty-nine years before. For years he had injected epinephrine hydrochloride in large amounts, using as much as a bottle, presumably 30 cc., in a week. Eosinophils constituted as much as 14 per cent of the white blood cells, and the same cells were found in large numbers in the sputum. In 1914, there was a penile sore and the Wassermann reaction was transiently 4 plus. In 1937 the Kline reaction of the spinal fluid was 4 plus. In 1934 a tumor of the bladder, diagnosed in another hospital as carcinoma, was removed; it recurred, but its growth was arrested by implantation of radon seeds and external application of radiation. He had experienced dyspnea on exertion and precordial pain of increasing severity for eighteen months, with orthopnea for eight months. Digitalis in 0.1 Gm. doses had been taken daily for one year.

The heart was enlarged 11 cm. to the left of the midline. The chest was barrel shaped, and the breath sounds were hyperresonant, with many sibilant and sonorous rales. The blood pressure was 164 systolic and 94 diastolic in 1934, 180 systolic and 90 diastolic two months before death and 130 systolic and 74 diastolic terminally. The urine contained a few red blood cells and many white blood cells.

While in the New York Hospital, the patient had transient heart block six weeks before death, which later reappeared. There was a sharp attack of epigastric pain to the right that lasted for one day and was accompanied by electro-



Hyaline changes in a medium-sized artery in the myocardium.

cardiographic changes indicating a lesion of a coronary artery. The man died after a period of increasing dyspnea.

At autopsy, the pericardium contained 40 cc. of clear yellow fluid. The heart weighed 450 Gm. and was firm. There were numerous fine translucent masses, about 1 mm. across, overlying the coronary arteries, "supra-arterial epicardial nodules" (Knox, J. H. M.: *J. Exper. Med.* **4**:245, 1899). The aorta contained vascular scars that were grossly and microscopically characterstic of syphilitic lesions. The medium-sized systemic and pulmonic arteries were moderately sclerosed on gross examination. The lungs were emphysematous and weighed 1,700 Gm. The peritoneum contained 400 cc. of clear fluid. The kidneys each weighed 150 Gm. Their surfaces were granular and light pink. The cortex was 4 mm. thick, the markings slightly indistinct. In the muscular wall of the bladder there were large areas of fibrosis, but no tumor was present on gross or on

microscopic study. The other abdominal organs were normal. There was a moderate amount of arteriosclerosis of the cerebral vessels, with slight cortical atrophy and slight internal hydrocephalus.

The medium-sized arteries of the heart, the adventitia of the aorta, the lungs, liver, gallbladder, spleen, kidneys, bladder and prostate, and the tissue around the adrenal glands showed advanced changes. In some vessels there was proliferation of the intima. The media was regularly greatly thickened. In many of the vessels from all organs there were clear areas between the remaining muscle fibers (figure) or even complete replacement of all structures with a hyaline material extending from the endothelium to the outer layers of the adventitia. This hyaline material was not found outside the walls of vessels except in small areas of the interstitial substance of the kidney, where it was surrounded by multinucleated giant cells.

The hyaline substance stained yellow with the Van Gieson stain and blue or olive green with the Mallory connective tissue stain. It never took stains characteristic of amyloid with congo red or with gentian violet. In paraffin sections cresyl violet gave no stain for amyloid, but a frozen section stained with cresyl violet and differentiated with acetic acid was brilliant red-violet. In frozen sections the clear material in the arterial walls stained bluish green with iodine before sulfuric acid was added. Dubarsch (*Virchows Arch. f. path. Anat.* **271**:867, 1929) found this reaction in what he described as "atypical amyloid." Reimann, Koncky and Eklund (*Am. J. Path.* **11**:977, 1935) found two types of deposits in the smooth muscles of the cardiovascular system, one reacting characteristically with cresyl and methyl violet and the other not reacting with them. Ritter (*Virchows Arch. f. path. Anat.* **192**:536, 1908), on the other hand, described widespread hyalinization in the walls of the smaller arteries without amyloid reactions.

In this case there is no direct evidence that syphilis was an etiologic factor. Although the findings are different in many respects from those in rabbits following injection of epinephrine hydrochloride (Josue: *Compt. rend. Soc. de biol.* **55**:1374, 1903; many others), the differences may be only those inherent in the different species. The possibility that the injections of epinephrine hydrochloride were an important etiologic factor cannot be denied in view of the long-continued, excessive parenteral injection of this drug. As stated, some staining reactions characteristic of atypical amyloid were observed.

DISCUSSION

AMOUR F. LIBER: The red staining with cresyl violet in the frozen section and its absence in the paraffin sections, together with the negative reaction with the ordinary stains for amyloid, is reminiscent of a substance found in the peripheral nerve fibers which has been called protagon and is presumably a lipoid akin to lecithin. I wonder whether there may not have been here some sort of lipoid analogous to protagon.

CHARLES T. OLCOTT: I cannot answer that question except to say that when the hyaline material was stained with sudan III there was no reaction for lipoid.

EPIDERMOID CARCINOMA OF THE BREAST. N. CHANDLER FOOT.

This paper has been submitted to the *American Journal of Cancer* for publication.

DISCUSSION

PAUL KLEMPERER: It is interesting that squamous cell carcinoma of the breast is not found much more frequently. In the last eleven years I have seen only 1 such neoplasm among 50,000 surgical specimens. I do not know exactly how many specimens of carcinoma of the breast are included in that number. It is also interesting that squamous metaplasia of the ducts is also rare. In the mamilla one encounters occasionally a growth originating definitely within the ducts which resembles closely basal cell carcinoma.

In regard to the classification of this tumor, there is no question. I am certain, however, a number of cases have been reported as instances of squamous cell

carcinoma which are in no way similar to that which Dr. Foot has presented. The diagnosis of squamous cell carcinoma should be reserved for tumors which actually show squamous cells with intracellular bridges and even hornification.

ARTHUR PURDY STOUT: My associates and I have had only 1 specimen of true squamous cell carcinoma of the mammary gland among some 850 specimens of carcinoma of the breast. This tumor pursued a course very similar to that which Dr. Foot has described. It was in an elderly colored woman, a tumor of short duration; she died eleven months after a radical mastectomy. No autopsy was made, but she probably had extensive metastases. We have had some patients —about 8—with carcinoma of the breast with squamous metaplasia, but I agree with both Dr. Foot and Dr. Klemperer that growths of this type should not be considered as true epidermoid carcinoma of the breast. Only such growths as that now described should be so classified.

EXPERIMENTAL HYPERTENSION. CHARLES G. CHILD (by invitation).

In 1934 Goldblatt and co-workers announced that significant hypertension could be produced in dogs by partial constriction of the renal arteries. It is my purpose in this paper to review several of the experiments on this recently discovered form of elevated arterial blood pressure undertaken in the department of surgery of the New York Hospital.

In each of 5 animals with experimental hypertension of this nature the spinal cord was destroyed below the level of the fifth cervical segment, and in another small group the renal arteries were not constricted until after the destruction of the cords. In the one group the established hypertension was not permanently reduced, and in the other the expected rise in blood pressure did not fail to appear. It was concluded that the neural pathways in the spinal cord were not fundamentally concerned in either the production or the maintenance of this type of experimental hyperpiesia.

In another group of experiments the extrinsic nerve supply of the kidney was studied. Although this had been previously investigated, the method of denervation seemed open to question. Eight animals were studied in which significant hypertension had been produced by partial constriction of the artery to a kidney transplanted to the femoral vessels. In all of these the normal kidney had been removed.

Based on the hypothesis that a substance capable of elevating the arterial blood pressure was present in the blood stream, two further experiments were made. In one the level of the guanidine bases was studied in both the acute and the chronic form of this morbid process. These substances were found in significantly increased amount whenever retention of nitrogen in the blood stream accompanied the elevation of blood pressure, but since similar rises were demonstrated in bilaterally nephrectomized animals, in which hypertension failed to appear, no relationship could be demonstrated between these substances and hypertension. In the second group of studies the effect of passing the return renal flow directly through the liver was observed. This was accomplished by means of a reverse Eck fistula. The results were negative; neither was established hypertension affected, nor did hypertension fail to appear when the arteries were constricted after the establishment of the fistula.

In conclusion, the pathologic changes found at autopsy in a series of animals, some with acute and some with chronic hypertension, are considered in considerable detail. In those in which death occurred within a week after constriction of the renal arteries the most constant observation was innumerable petechial hemorrhage, diffuse in distribution, predominating in the gastrointestinal tract and cardiac musculature. Although all of these animals died in uremia, the intensity of the lesions was far greater than would be associated with uremia alone. The conclusion is suggested that degenerating renal tissue, if absorbed, is effective in elevating the arterial blood pressure and profoundly affecting the capillary bed. In those dogs in which various stages of persistent hypertension

had been present for from one to ten months there was constantly found evidence of renal damage similar to that originally described by Goldblatt and his associates. Of further interest was the frequency with which rather marked degrees of arteriolar sclerosis were noted after the blood pressure had been elevated for from three to four months or longer. Although these studies have failed to elucidate the mechanism of the initiation of this type of hypertension, the arterial disease found in its later stages was suggested as offering a possible explanation for its maintenance.

MYOCARDIAL HYPERTROPHY OF UNCERTAIN CAUSE, ASSOCIATED WITH CONGESTIVE HEART FAILURE: THE ROLE OF ANTECEDANT HYPERTENSION. BERNARD I. KAPLAN (by invitation), EUGENE CLARK and CLARENCE E. DE LA CHAPELLE.

A study of 43 cases of congestive heart failure, with preponderant left ventricular hypertrophy of uncertain cause, is reported. Valvular disease, congenital cardiac defects, syphilitic aortitis, adhesion of the pericardium and inflammatory myocardial disease were absent, and the available blood pressure readings were normal. The possible role of antecedent hypertension in the development of cardiac hypertrophy in these patients was considered by comparing the incidence of renal arteriolar sclerosis in this group with that in a group of 154 hypertensive and 269 nonhypertensive persons.

Mild or severe renal arteriolar sclerosis was found in 12.6 per cent of nonhypertensive patients, increasing from an incidence of 5.1 per cent in the fourth decade to 42.8 per cent in the ninth. Of the patients with hypertension who died of congestive heart failure, 82.5 per cent showed mild or severe renal arteriolar sclerosis, whereas only 54.6 per cent of the patients with hypertension in whom death was not caused by congestive heart failure, uremia or cerebral vascular disease showed significant renal arteriolar sclerosis. Only 30.9 per cent of the patients who had congestive heart failure with cardiac hypertrophy of uncertain cause showed renal arteriolar sclerosis, suggesting that arterial hypertension had not existed in most of the persons of this group.

An effort was made to analyze further the factors which might have operated in the production of cardiac hypertrophy in these patients. In 18 of them myocardial infarction was present, and because they showed an incidence of renal arteriolar sclerosis of 39 per cent, it is felt that there had been antecedent hypertension in many. Of the remaining 25 patients, renal arteriolar sclerosis was found in 6, and all these were in the seventh and eighth decades of life. In 10 of the latter group other factors were found which may have operated in the genesis of cardiac hypertrophy, e. g., coronary sclerosis, protracted auricular fibrillation, chronic anemia and hyperthyroidism; in the remaining 15, structural or functional abnormalities which might have initiated cardiac dilatation and hypertrophy were not apparent. It is felt that, although the role of antecedent hypertension cannot be excluded in every case, its absence in the majority of these cases appears probable.

DISCUSSION

HERBERT J. WIENER: A case I encountered some years ago is of interest, more particularly from the clinical side. Dr. Kaplan spoke of the difficulty of proving that there had previously been hypertension, a difficulty which is commonly encountered in hospital work; one does not know the antecedent clinical history.

This patient was a man of 51 years who died in 1930. I had followed the clinical course of his illness at frequent intervals since 1921, a period of a little over nine years. He died of coronary thrombosis with infarction of the ventricle. There was never any indication of hypertension, and records of his blood pressure were made at frequent intervals throughout these nine years, including the final illness. The clinical features of the terminal cardiac failure were perfectly typical of coronary thrombosis. A partial autopsy was done by Dr. C. R. Rabin, and Dr. Klemperer saw the material. The extreme hypertrophy of the cardiac muscle with this clinical history and with no evidence of renal damage clinically and, as

it developed, little evidence pathologically evoked comment. The heart was exceedingly heavy as compared with the size of the patient. I cannot give the exact weight of the heart, but I remember it was between 600 and 700 Gm., and the man was 5 feet 7 inches tall (170 cm.) and not obese. Although the kidneys were smooth grossly and not contracted, microscopic sections showed unmistakable evidence of a moderate degree of arteriolar sclerosis. There was some evidence of this in the pancreas as well. Microscopic sections of the cardiac muscle, as in the cases described by Dr. Kaplan, showed nothing peculiar other than the changes seen in the hypertrophied myocardium in the typical hypertensive group.

The kidneys were reported to be somewhat larger than normal; the liver was considerably enlarged; the spleen was somewhat enlarged and firm; the adrenal cortices were about twice the normal width.

The possibility of an acromegaloid enlargement of all the viscera might be entertained.

ROBERT A. MOORE: May I ask whether Dr. Kaplan made any observations concerning the thickened basement membrane of the glomerulus which has been observed in essential hypertension, especially with the use of the modification of the Heidenhain-Mallory stain?

I should also like to inquire concerning the possible significance of the small foci of fibrosis which he described in the myocardium. Does he consider it possible that myocarditis—for example, Fiedler's myocarditis—could heal and thus result in a hypertrophic condition of the heart which would not otherwise be explained?

PAUL KLEMPERER: I am amazed at the percentage of only 82 for arteriolar hyalinization in hypertension. In the past twenty years a rather large number of reports have appeared, and if I am correct in my recollection of them and also of my own experience, the percentage was always above 90. The last report of Moritz and Oldt was 96 or 97 per cent. Eighty-two per cent is really low, and it may be significant. As long as one has to rely on arteriolar sclerosis in hypertension, as an indicator, one must realize that a certain percentage of the patients do not show it. Eighty-two per cent is unusually low, so that I wonder whether this can be explained by the fact that the hypertension did not run its prolonged course. I recall that in my own cases of hypertension in young people who died suddenly, not of cardiac insufficiency but of intercurrent disease, a cerebral accident or some other cause, arteriosclerosis of severe grade was found.

BERNARD I. KAPLAN: In reply to Dr. Moore: We made no studies of thickening of the basement membrane. We did not consider the foci of myocardial fibrosis as evidence of healed interstitial myocarditis, first, because they were sparse and small, most of them being of only microscopic size; second, they were most commonly perivascular in distribution, and third, they were entirely similar to foci encountered by us in an independent study of hearts hypertrophied from various causes, including hypertensive hearts as well as hearts like these.

In reply to Dr. Klemperer, I cannot do much more than stress that the figure of 82.5 per cent represents the incidence of renal arteriolar sclerosis in that group of patients with essential hypertension who died of congestive heart failure. In patients with hypertension who died of cerebral vascular disease or of uremia, the incidence was much higher, so that if we combined all three groups, as Moritz and Oldt did, we should obtain a figure approaching theirs, but for the group who died of congestive heart failure alone, the incidence is somewhat less. Another point of difference from other published series may be that we excluded entirely from consideration sparse lesions of arteriolar sclerosis because we encountered them so commonly and with such constancy in all groups that we felt they had no relation to hypertension or to age.

As far as the incidence of arteriolar sclerosis in the kidneys in the control group is concerned, it is much less, being only 12.6 per cent, compared with the total incidence given by Moritz and Oldt, which is 12 per cent. We showed the distribution by age in decades merely to bring out an additional point.

Book Reviews

Esperimenti di vaccinazione antitubercolare. Eseguiti sotto il controllo del Comitado Tecnico-Scientifico dell'Istituto Vaccinogeno Antitubercolare. Relazione Stesa dal Prof. Alberto Ascoli. Presentazione di S. E. Sante De-Blasi, Presidente del Comitato per la Medicina del Consiglio Nazionale delle Ricerche. Paper. Milan: Laboratori di patologia Comparata della Regia Universita e Istituto Vaccinogeno Antitubercolare di Milano, 1936.

This book is a carefully documented account of a study on the relative practical values of BCG and heat-killed tubercle bacilli in prophylaxis against tuberculosis in cattle. The data reported in this volume seem to justify the conclusions reached by the author, within the scheme of the conducted experimentation.

The experiments were made on ninety-four calves. By intravenous or subcutaneous treatment with living BCG or human tubercle bacilli calves could be protected against inoculation of virulent tubercle bacilli, without the development of tuberculous lesions; this was demonstrated when they were killed long after all the controls had died of acute miliary tuberculous pneumonia. The treatment was more effective if the vaccinations were repeated. If the infecting dose exceeded ten times the dose that was lethal for the controls, or if too long an interval elapsed between vaccination and the test, no protection was evident in the vaccinated animals. But even with moderate infecting doses, if the calves were killed more than seven months after inoculation, the vaccinated animals showed regressive tuberculous lesions.

Vaccination with heat-killed BCG or human tubercle bacilli, even when performed with large amounts and repeated at intervals, never prevented the development of tuberculous lesions, and, although the vaccinated calves survived the inoculation of bacilli virulent for the controls, when exposed to natural contagion, they were not protected, even when the contagion was so low that only one of four unvaccinated controls acquired tuberculosis. The calves vaccinated with heat-killed bacilli were not protected against natural contagion even when fed with tubercle bacillus-free milk and housed in quarters free from tuberculosis during the process of vaccination. If the calves were not protected from contagion during the process of intravenous vaccination with heat-killed tubercle bacilli, the incidence of tuberculous lesions among them was three times more frequent than among the controls. Under the same conditions, the incidence of tuberculous infection in calves vaccinated with living BCG was not greater than that among the controls. However, if the calves were protected from contagion during the process of vaccination with living BCG, they exhibited marked protection to stable infection, so that in one experiment in which the exposure was so intense that all the calves treated with heat-killed tubercle bacilli, as well as the unvaccinated controls, showed tuberculous lesions when slaughtered, four of five cattle vaccinated with living BCG were entirely free from lesions, and the excepted animal revealed only an isolated tuberculous mesenteric lymph node. If the intensity of the contagion was reduced under these conditions, BCG-vaccinated cattle were completely protected, while three of four controls contracted tuberculosis. The committee recommends the use of the living BCG vaccine and emphasizes the importance of protecting the calves from contagion during the process of vaccination.

The laborious character of these studies, as well as the prohibitive expense involved, preclude more extensive and more varied trial of different forms of vaccination with heat-killed tubercle bacilli both as to the preparation of the antigen and as to its mode of administration.

The book is of value to public health workers and pathologists for the wealth of detail and the careful scrutiny with which the data are evaluated.

Some Fundamental Aspects of the Cancer Problem. Symposium Sponsored by the Section of Medical Sciences of the American Association for the Advancement of Science, Atlantic City, New Jersey, December 29, 1936-January 1, 1937. Edited by Henry Baldwin Ward. Publication Committee: William Charles White, chairman; Clarence Cook Little, Esmond R. Long, Vincent du Vigneaud, Carl Voegtlin. Cloth. Pp. 248, with illustrations. Price, $2.50. New York: The Science Press, 1937.

At the annual meeting of the American Association for the Advancement of science in Atlantic City, Dec. 29, 1936, to Jan. 1, 1937, thirty-one persons, eminent in the various branches of cancer research, presented papers in a symposium on cancer. These papers have been gathered together and published in book form essentially as they were given at that meeting. They represent the most varied branches of scientific investigation, as for instance the genetics of cancer, the relation of carbohydrate metabolism to cancer and the bearing of the latest discoveries in atomic physics. As one reads this book one becomes aware that there is probably no person alive who would be capable of intelligent detailed criticism of all the papers presented. That this is so shows how complex and elusive the solution of the problem of cancer appears to be at present.

The papers were presented in sections dealing with the following phases of the problem: heredity and constitutional factors; induction, stimulation and inhibition of tumorous growths; metabolism of cancerous tissue, and the effects of radiation. Finally, there is a discussion of the cancer problem in general.

Under the first subtitle, "Heredity and Constitutional Factors," are grouped papers by: Maud Slye; C. C. Little; Clara R. Lynch; W. F. Dunning, M. R. Curtis and F. D. Bullock; A. M. Cloudman, J. J. Bittner and C. C. Little; E. O. MacDowell and Felix Bernstein. Miss Slye presents evidence in support of the theory that cancer is the result of mendelian inheritance of a recessive character for the type of tumor combined with a recessive character for its location. Little gives data to show that the so-called inheritance of mammary cancer in mice probably is determined by an extrachromosomal factor transmitted through the maternal line. Lynch's paper is logical and clearly written. Her results suggest that the occurrence of cancer of the lung in mice is controlled by a semidominant gene. She shows also that susceptibility to tumors of the skin following tarring is inherited, but independently from that to tumors of the lungs in the same animals. Dunning, Curtis and Bullock show with cysticercus sarcoma in the rat and with tumors produced with 1,2,5,6-dibenzanthracene and with 3,4-benzpyrene, given in paraffin pellets, that the probability of cancer formation is unrelated to the genetic constitution of the animal but is directly proportional to the number of cancerigenic foci (cysts or paraffin pellets). Cloudman, Bittner and Little studied the occurrence of spontaneous tumors in three pure strains of mice, and in their paper they give the incidence of these tumors in various organs for each strain. Unfortunately there is a misprint in the first table: the column labeled "A" stock should be labeled "B" stock. They point out that if the presence of cancer depends on a single gene, all homozygous stocks in which cancer occurs and which are kept under identical environmental conditions should show identical incidence of tumors. This they obviously do not show. MacDowell experimented with homozygous strains of mice in which leukemia occurred spontaneously. His figures fail to show either dominance or recessiveness of this character in the F generations. He suggests that the occurrence of leukemia is due to a combination of hereditary, or "intrinsic," factors with environmental, or "extrinsic," factors. He believes that homozygous animals offer an opportunity for the study of the important extrinsic factors and thus an avenue to the determination of measures preventive of human cancer. Bernstein suggests that Lynch's figures may be explained on the assumption that the inheritance of cancer of the lung in mice is recessive below the age of 24 months and dominant thereafter.

The conclusion one draws from reading these papers is that although heredity undoubtedly plays a role in the incidence of tumors in animals the matter of the

exact manner of inheritance and that of whether or not this follows mendelian laws are still far from settled.

The second section is presented under the subtitle "Induction, Stimulation, and Inhibition of Tumorous Growth." It contains papers by: L. F. Fieser; F. C. Wood; H. B. Andervont; W. U. Gardner; C. F. Geschickter and E. B. Astwood; R. S. Ferguson; H. J. Bagg; J. B. Murphy, and J. W. Jobling, E. E. Sproul and S. Stevens. Fieser discusses the relationship of chemical structure to carcinogenesis. His researches have led him to the conclusion that the alkyl substituent in the 10 position of the 1,2-benzanthracene complex accounts for the high carcinogenic potency of methylcholanthrene rather than the presence of a cyclopenano ring in this compound. Wood gives a short summary of the chemical methods of producing tumors. Andervont shows that subcutaneous injection of 1,2,5,6-dibenzanthracene increases the incidence of spontaneous tumors in the lungs of mice and even produces such tumors in strains in which the tumors ordinarily never develop. It is suggested that these tumors may arise by direct action of the absorbed hydrocarbon in the circulation. Gardner finds that cancer seldom develops in male mice receiving small doses of estrogen, though marked development of the breasts results, while, of male mice of three different strains receiving 500 international units of keto-estrin benzoate (3-benzoate-17-keto-1,3,5-estratriene) or of hydroxy-estrin benzoate (3-benzoate-17-hydroxy-1,3,5-estratriene) weekly, mammary cancer developed in 80 per cent. These results suggest that mammary cancer in the male mouse results from abnormal stimulation of mammary tissue rather than from normal development of this tissue in an animal susceptible to cancer. Geschickter and Astwood compare the changes produced in the human breast by the injection of estrogenic substances with those produced in experimental animals. Ferguson has examined 290 specimens of teratoma testis, 80 of which were made into serial sections. He finds that 60 of the tumors contained tissues derived from the three germ layers and that the age at which these tumors occurred was approximately 25 years; 120 of the tumors were homogeneous in structure, and these occurred at an average age of 36 years; the remaining 110 were intermediate in type; 10 showed no relationship to the rete testis. He concludes that teratoma testis is a tumor arising from the germ cell no matter where this may be, that the character of the tumor depends somewhat on the age of the host and that in every case it arises originally from a totipotent cell. Bagg presents his results in repeating the work of Michalowsky on the production of teratoma testis in the fowl with zinc chloride. Murphy has continued his search for substances inhibiting and stimulating tumor growth, previously carried out on chicken sarcoma tissue and placenta and embryonic skin. He finds that the mammary gland in the prelactating or early lactating stages contains large amounts of inhibiting and stimulating substances. The former are ether insoluble, while the stimulating fractions are ether soluble. Jobling, Sproul and Stevens have found that a lipid extracted from Rous chicken sarcoma with either carbon tetrachloride or purified petroleum benzene (petroleum ether) in vacuo at low temperatures will reproduce the tumor when mixed with an extract of normal chicken tissue or with a solution of casein. The extracted lipid does not contain protein as determined by anaphylactic reactions. The most significant papers in this section are probably those of Fieser, Gardner, Murphy and Jobling and his associates.

The section on the metabolism of cancerous tissue contains papers by: W. H. Lewis; Dean Burk; C. Voegtlin, J. M. Johnson, M. E. Maver and J. W. Thompson; F. S. Hammett, and G. H. Scott. Lewis reiterates his previous statements that tumor cells can be recognized in tissue culture by their morphologic and cultural characteristics. Burk reviews at some length the work that has been done on the problem of carbohydrate metabolism. He describes Warburg's work, the Pasteur-Meyerhof cycle and Lipmann's modification of Meyerhof's theory. This is decidedly a paper comprehensible only to those who are thoroughly familiar with the field. Voegtlin, Johnson, Maver and Thompson have shown that an adequate supply of lysine, tryptophan and cystine in the diet is essential for the

growth of spontaneous mammary cancers in the Marsh strain of mice. When the cystine content of the diet is low, the addition of glutathione causes a marked increase in the rate of tumor growth. Hammett reports experiments on Obelia to determine the effects of the different amino-acids in varying concentration on the growth and maintenance of the organisms. Scott has compared embryonic and cancerous tissues with homologous adult tissues by the micro-incineration method of Policard. He concludes that, while in adult cells there is a decidedly constant pattern of mineral distribution for each tissue, in embryonic and in cancerous tissue there is a continual shift in the quality and quantity of the mineral salts.

The section on radiation contains papers by: M. A. Tuve; L. S. Taylor; G. Failla; J. H. Lawrence, P. C. Aebersold and E. O. Lawrence; R. E. Zirkle; J. J. Jares Jr. and S. L. Warren, and R. D. Evans and J. C. Aub. Tuve discusses in a somewhat general fashion some of the properties of gamma rays developed at very high voltages, the possibility of using very fast beta particles in biologic experimentation, positive ion rays, neutrons and artificial radioactivity. Taylor presents some of the results of his prolonged efforts to define in a practical manner the quality of a roentgen ray beam. Failla sets forth the theory that the biologic effects of radiation are caused by an increase in the number of nondiffusible intracellular and intranuclear ions, with a resulting change in osmotic relationships, plus a weakening of the cell membrane by direct ion hits, rather than by chemical changes in the colloids of the cell. The Lawrences have compared the effects of neutrons, obtained from the cyclotron with roentgen rays, on transplantable mammary carcinoma in the mouse, on Drosophila eggs and on normal mice irradiated in toto. The damaging effects of neutrons on the body as a whole are similar qualitatively to those of roentgen rays. Mammary carcinoma of the mouse is more susceptible to the effects of neutrons than to those of roentgen rays when compared with Drosophila eggs or entire mice.

Zirkle has shown, by using alpha particles from polonium and the nucleus of the spore of the fern *Pteris longifolia*, that the more closely ions are spaced along the track of an alpha particle the greater is their biologic effectiveness. He argues from this that the biologic effectiveness of neutrons should be greater than that of roentgen rays per ion produced and should be a little more than a fifth of that of alpha particles at the ends of their paths. Jares and Warren, using the Brown-Pearce rabbit carcinoma, have shown that the combination of fractional roentgen therapy and fractional fever therapy in vivo gives approximately double the percentage of local cures of either type of therapy alone. Evans and Aub studied the radium excretion of three patients suffering from radium poisoning. Their metabolic studies were carefully carried out and well controlled, and their determinations of radioactivity were made with sensitive Geiger counters. They conclude that the excretion of radium from the body can be increased by methods which mobilize calcium but that this increase is more marked when the radium poisoning is in the early than when it is in the chronic stage.

The last section, given to a general discussion of the cancer problem, contains only three papers. They are by L. I. Dublin, C. C. Little and Walter Schiller. Dublin reviews and analyzes the mortality statistics of the Metropolitan Life Insurance Company from 1911 to 1935 and concludes that when corrections are made for the increasing longevity of the population there is little evidence that cancer as a whole is increasing; some forms of cancer show a definite decline in rate, such as cancer of the female generative organs, of the buccal cavity and of the skin. Cancer of the lungs, of the pancreas and of the prostate shows a tendency to increase. Little points out the benefits to be derived from organized efforts for the control of cancer. Schiller's paper contributes little but reemphasizes his theory that cancer cells turn normal cells into cancer cells by contact with them.

On the whole, a collection of papers such as this is rather disappointing. Most of the papers are too short for detailed presentation of the material or of the experimental data. Their publication in book form makes them more inaccessible to those working in the special fields in which the knowledge would be useful

if they were published in the appropriate current journals. The information contained in the book is too specialized to be of use even to the man engaged solely in cancer therapy and is not detailed enough for the trained laboratory worker in cancer research. However, as a means of commemorating an interesting occasion, the publication of the book was probably worth while.

A Monograph on Veins. By Kenneth J. Franklin, D.M., M.R.C.P., Tutor and Lecturer in Physiology, Oriel College; University Demonstrator of Pharmacology and Assistant Director of the Nuffield Institute for Medical Research, Oxford. Cloth. Price, $6. Pp. 410, with 46 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

The author of this, the first English monograph on veins, states in his preface that his "object in writing the book was to make available to others a somewhat recondite literature, which only a specialist could hope to summarize but which has, nevertheless, very definite bearings upon physiological, pathological and clinical problems." He has not only attained this object in a satisfying manner but has added original observations and important conclusions of his own that greatly increase the value of the book. His qualifications for writing such a monograph are indicated by the fact that in the comprehensive bibliography, occupying 53 pages, there are 29 articles of which he is the senior author.

The first chapter of the monograph is an interesting historical introduction. The only chapter not contributed by the author is that on the embryology of veins, written by Keith Richardson. The variations in anatomic structure of different veins are shown to be related to their functions. Three chapters deal with veins and the nervous system, with a discussion of the effects of stimulation by various chemical substances, including glandular preparations; others, with the heart and venous return, respiration and venous return, and venous pressure. In the chapter tersely entitled "Clinical." there is much useful and practical information on intravenous injection, varicose veins, thrombosis, phlebitis, air embolism and other subjects. A chapter on the application of various photographic technic to research on the venous system presents new work on infra-red photography and x-ray cinematography.

The title, "A Monograph on Veins," suggests a highly technical presentation of a very small area of medical science. But by considering veins from almost every conceivable point of view, the author has brought together a mass of well documented information in which the anatomist, the physiologist, the pharmacologist, the pathologist, the endocrinologist and the clinician will each find something of interest. The volume contains 350 pages of text, 46 illustrations and 60 pages of bibliography and index.

The literature on veins is discussed critically by one thoroughly competent to judge the accuracy of statements of facts and to evaluate the conclusions of the authors cited. He has formulated definite opinions concerning the various phases of the subject and states his conclusions clearly and concisely. Both in the preface and in the final chapter he modestly declares that "Most conclusions are, in any case, temporary and even mistaken ones will not seriously impede, for any length of time, that progress of research, which it is the primary object of this book to aid." This volume can be confidently recommended as a comprehensive and discriminating survey of the accumulated knowledge on veins.

Books Received

REPORT OF THE DIRECTOR OF THE SCHOOL OF TROPICAL MEDICINE OF THE UNIVERSITY OF PUERTO RICO UNDER THE AUSPICES OF COLUMBIA UNIVERSITY FOR THE YEAR ENDING JUNE 1937. Paper. Pp. 67. San Juan: University of Puerto Rico and Columbia University, 1937.

TEXT-BOOK OF EXPERIMENTAL SURGERY. Jacob Markowitz, M.B., Ph.D., M.S., Research Associate, Department of Physiology, University of Toronto. Cloth. Price, $7. Pp. 527, with 330 illustrations. Baltimore: William Wood & Company, 1937.

FEVER THERAPY. ABSTRACTS AND DISCUSSIONS OF PAPERS PRESENTED AT THE FIRST INTERNATIONAL CONFERENCE ON FEVER THERAPY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, NEW YORK CITY, MARCH 29, 30, 31, 1937. Edited by the Members of the American Committee; Dr. Walter M. Simpson, Dayton, Ohio, Chairman. Cloth. Price, $5. Pp. 486. New York: Paul B. Hoeber, Inc., 1937.

DE L'INFLUENCE DE DIVERS CATIONS SUR LE CROIT MICROBIEN. ÉTUDE DE L'ACTION, A DOSES DIVERSES, DU LANTHANE, DU CERIUM, DU PLOMB, DU MERCURE, DE L'ARGENT, SUR LA MULTIPLICATION DU BACILLE PYOCYANIQUE. RÔLE DES CHARGES ELECTRIQUES. Lucien Neipp, docteur es sciences pharmacien de l'Université de Paris, ancien moniteur de bacteriologie à la Faculté de pharmacie. Paper. Price, 90 francs. Pp. 430. Paris: Masson & Cie, 1937.

LE PHÉNOMÈNE DE LA GUÉRISON DANS LES MALADIES INFECTIEUSES. F. d'Herelle. Paper. Price, 75 francs. Pp. 416, with 4 illustrations. Paris: Masson & Cie, 1938.

A CLINICAL AND GENETIC STUDY OF 1280 CASES OF MENTAL DEFECT. L. S. Penrose. Medical Research Council Special Report Series, no. 229. Paper. Price, 2 shillings, sixpence. Pp. 159. London: His Majesty's Stationery Office, 1938.